1986

Seventh Biennial Cheese Industry Conference

Various Authors

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7TH BIENNIAL
CHEESE INDUSTRY CONFERENCE
AUGUST 26-28, 1986

PARTICIPANT LIST

UTAH STATE UNIVERSITY
LOGAN, UTAH
<table>
<thead>
<tr>
<th>Name</th>
<th>Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doug Anker</td>
<td>KRAFT INC 801 WAUKEGAN ROAD GLENVIEW IL 60025</td>
</tr>
<tr>
<td>Brent Ball</td>
<td>CHEMAX DIV. 1987 SOUTH 700 WEST Salt Lake City UT 84104</td>
</tr>
<tr>
<td>Gerald Barnes</td>
<td>MID-AMERICA DAIRYMEN 319 N 72ND STREET OMAHA NE 68114</td>
</tr>
<tr>
<td>Al Bauer</td>
<td>112 WEST FAIRVIEW DR MARSFIELD WI 54449</td>
</tr>
<tr>
<td>Donald Benning</td>
<td>MID AMERICA DAIRYMEN 800 WEST TAMPA SPRINGFIELD MO 65805</td>
</tr>
<tr>
<td>Poul Bjerre</td>
<td>DENMARK 84322</td>
</tr>
<tr>
<td>Bob Blanck</td>
<td>850 MAIN STREET WILMINGTON MA 01887</td>
</tr>
<tr>
<td>Paul Bokelman</td>
<td>196 WESTERN AVE FONDULAC WI 54935</td>
</tr>
<tr>
<td>Lawrence Brennan</td>
<td>Associated Milk Prod Route 3 HILLSBORO KS 67063</td>
</tr>
<tr>
<td>Ean Brockwell</td>
<td>ALFA-LAVEL 2950 METRO DRIVE #20 BLOOMINGTON MN 55420</td>
</tr>
<tr>
<td>David Brown</td>
<td>Dairy Specialties In PO Box 594 Worthington WA 43085</td>
</tr>
<tr>
<td>Judith Aulik</td>
<td>122 ECKLES HALL UNIV OF MISSOURI American Fork UT 65201</td>
</tr>
<tr>
<td>Lonnie Banning</td>
<td>1005 N PENBROKE CT EFFINGHAM IL 62401</td>
</tr>
<tr>
<td>Norman Battenberg</td>
<td>LUGANO CHEESE CO BOX 485 MONROE WI 53566</td>
</tr>
<tr>
<td>Jeffery Bell</td>
<td>Kraft Inc. 4710 Chateau St Pocatello ID 83202</td>
</tr>
<tr>
<td>Charles Berigan</td>
<td>KRAFT INC KRAFT COUR GLENVIEW IL 60025</td>
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<tr>
<td>Lee Blakely</td>
<td>DIARYMAN'S 400 SOUTH M STREET TULARE CA 93274</td>
</tr>
<tr>
<td>Floyd Bodyfelt</td>
<td>OREGON STATE UNIVERS DEPT FOOD SCIENCE &amp; CORVALLIS OR 97331</td>
</tr>
<tr>
<td>Fran Brady</td>
<td>196 WHIPPLE ROAD TEWKSBURY MA 01876</td>
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<tr>
<td>Donald Brick</td>
<td>SWISS VALLEY FARMS P O BOX 4493 DAVENPORT IA 52808</td>
</tr>
<tr>
<td>Gordon Brown</td>
<td>196 WESTERN AVE. FONDULAC WI 54935</td>
</tr>
<tr>
<td>Robert Buchheim</td>
<td>LAND O LAKES INC. 526 HENRY AVENUE VOLGA SD 57071</td>
</tr>
</tbody>
</table>
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Johnnie Nichols
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200 C ST S W
WASHINGTON DC
20204
<table>
<thead>
<tr>
<th>Name</th>
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<tr>
<td>Scott Nickell</td>
<td>MILES LABORATORIES</td>
<td>Roy</td>
<td>UT</td>
<td>84067</td>
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<td>K.m. Nilson</td>
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<td>Campbell</td>
<td>NY</td>
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<td>Thomas Otto</td>
<td>320 NORTH MAIN St Peter</td>
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<td>MN</td>
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<td>Bloomington</td>
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<td>CA</td>
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<td>MEAD JOHNSON</td>
<td>Evansville</td>
<td>IN</td>
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<td>Mike Rice</td>
<td>DAIRYMANS COOP CREAM</td>
<td>Tulare</td>
<td>CA</td>
<td>93274</td>
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<td>John Rothenbuhler</td>
<td>MIDDLEFIELD CHEESE</td>
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<td>Joseph Ruocco</td>
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<td>Denver</td>
<td>CO</td>
<td>80222</td>
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<td>Petaluma</td>
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<td>Wc Nielsen</td>
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<td>Dawson</td>
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<tr>
<td>Joseph Peterson</td>
<td>500 SOUTH LINCOLN AV</td>
<td>Marshfield</td>
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<tr>
<td>Mali Reddy</td>
<td>AMERICAN DAIRY &amp; FOOF</td>
<td>Denver</td>
<td>CO</td>
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<td>Rob Riber</td>
<td>GALLOWAY WEST COMPANY</td>
<td>Alfred Station</td>
<td>NY</td>
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<td>Gary Ring</td>
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<td>Fondulac</td>
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<td>Leonard Ruiz</td>
<td>LAND O'LAKES</td>
<td>Minneapolis</td>
<td>MN</td>
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<tr>
<td>Bill Sandine</td>
<td>OREGON STATE UNIVERS DEPT OF MICROBIOLOGY</td>
<td>Corvallis</td>
<td>OR</td>
<td>97331</td>
</tr>
<tr>
<td>Ron Schmidt</td>
<td>3920 SW 4TH PL</td>
<td>Gainesville</td>
<td>FL</td>
<td>32607</td>
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<tr>
<td>Robert Sellars</td>
<td>Chr. Hansens Lab.</td>
<td>Milwaukee</td>
<td>WI</td>
<td>53214</td>
</tr>
</tbody>
</table>
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# CHEESE INDUSTRY CONFERENCE SCHEDULE

**TUESDAY, AUGUST 26, 1986**

**THEME:** OUR INDUSTRY TODAY AND TOMORROW  
Chairman: Delores Wheeler

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Presenter</th>
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</thead>
<tbody>
<tr>
<td>9:00-10:00</td>
<td>Registration, Eccles Conference Center Registration Booth</td>
<td>Doyle Matthews</td>
</tr>
<tr>
<td>10:00-10:15</td>
<td>Utah State University Welcomes You</td>
<td>Joe Lyon</td>
</tr>
<tr>
<td>10:15-10:45</td>
<td>New dairy promotion and research programs</td>
<td>Hollis Hatfield</td>
</tr>
<tr>
<td>10:45-11:25</td>
<td>Effect of the buy-out and future government programs on milk supplies</td>
<td>John Siebert</td>
</tr>
<tr>
<td>11:25-12:00</td>
<td>Present and future status of west coast cheese markets</td>
<td>Peter Linklate</td>
</tr>
<tr>
<td>12:00-1:00</td>
<td>Lunch, Carousel Square, University Center (on your own)</td>
<td>John W. Courtright</td>
</tr>
</tbody>
</table>

**Afternoon Session**

**THEME:** CULTURES AND ENZYMES  
Chairman: John W. Courtright

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Presenter</th>
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<tbody>
<tr>
<td>1:15-1:55</td>
<td>A unique system for enhancing Cheddar cheese aging</td>
<td>Peter Freund</td>
</tr>
<tr>
<td>1:55-2:35</td>
<td>pH controlled starter: A decade later</td>
<td>Randall Thune</td>
</tr>
<tr>
<td>2:35-3:10</td>
<td>Genetic engineering: What it can do for starter cultures</td>
<td>Jeffery Kondo</td>
</tr>
<tr>
<td>3:10-3:25</td>
<td>Snacks and Conversation</td>
<td></td>
</tr>
<tr>
<td>3:25-4:15</td>
<td>Cheeses from new technology--Are they traditional?</td>
<td>Merrill Thompson</td>
</tr>
<tr>
<td>4:15-4:50</td>
<td>Effect of proteinase negative starter cultures on curd yields</td>
<td>Gary Richardson</td>
</tr>
<tr>
<td>6:00</td>
<td>Leave for Malibu/Guinivah Picnic Site, Logan Canyon</td>
<td></td>
</tr>
<tr>
<td>6:30</td>
<td>Steak Fry Served</td>
<td>Malibu/Guinivah Picnic Site, Logan Canyon</td>
</tr>
</tbody>
</table>

**WEDNESDAY, AUGUST 27, 1986**

**THEME:** INNOVATIVE TECHNOLOGIES  
Chairman: Douglas Larson

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Presenter</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:30-9:10</td>
<td>Computerized process control for Cheddar cheese manufacture</td>
<td>Peter Linklate</td>
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<td>9:10-9:45</td>
<td>Linear programming in the allocation of milk resources for cheese making</td>
<td>Gary Kerrigan</td>
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<td>9:45-10:20</td>
<td>Continuous curd formation in cheese manufacture</td>
<td>Nicholas Darras</td>
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<td>10:20-10:35</td>
<td>Snacks and Conversation</td>
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<td>10:35-11:10</td>
<td>Direct acid cottage cheese from ultrafiltered skim milk</td>
<td>C. A. Ernstrom</td>
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<td>11:10-11:50</td>
<td>Predicting shelf life of cottage cheese by impedance microbiology</td>
<td>Charles White</td>
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<td>12:00-1:00</td>
<td>Lunch, Carousel Square, University Center (on your own)</td>
<td>Johnnie Nichol</td>
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**Afternoon Session**

**THEME:** QUALITY TRENDS AND CONCERNS  
Chairman: Keith Geilman

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<th>Time</th>
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<tr>
<td>1:15-1:55</td>
<td>FDA concerns about cheese and cheesemaking procedures</td>
<td>Johnnie Nichol</td>
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<tr>
<td>1:55-2:25</td>
<td>Yes, you can make good quality cheese from fully</td>
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pasturized milk

2:25- 3:05  Effect of milk psychotrophs on flavor, body, and chemical profiles of Cheddar cheese  Robert Lowrie

3:05- 3:20  Snacks and Conversation  Charles White

3:20- 3:55  Improved cheese yields by controlling psychotrophs in raw milk  Robert Sellars

3:55- 4:25  Tracing cheese defects to their source  Robert Olsen

4:25- 5:00  Cheddar cheese: Who should be the judge?  Floyd Bodyfelt

THURSDAY, AUGUST 28, 1986

THEME: WHEY AND PERMEATE UTILIZATION  Chairman: Clint Warby

8:30- 9:10  Status of whey and whey products today  Warren Clark

9:10- 9:45  Does it pay to make alcohol from whey  John Murtagh

9:45-10:00  Snacks and Conversation  

10:00-10:40  Another approach to single cell protein from permeate or whey  William Boon

10:40-11:15  It works for packing plant waste--could it work for whey or permeate?  Conly Hansen

11:15  Adjourn
OUR

INDUSTRY

TODAY

AND

TOMORROW
The topic I have been assigned is timely. I doubt, however, that I can add much to what many of you already know, but perhaps the way I package my comments will supplement your perspective.

The setting today is one of surplus and the central question being asked throughout the United States is how do we maintain a reasonable balance between the market requirements and the milk supply. My comments will be restricted to four areas: Herd Buyout; Some Supply Factors; Government Programs; and Mandatory Controls and the Cheese Industry.

**Herd Buyout**

The dairy herd buyout program will remove some 12 billion pounds of milk over an 18 month period. But the balance of the resources -- some 90 percent -- will not remain static. The relatively favorable milk-feed price ratio, the increasing production per cow, and the talk about quotas is generating a supply that is offsetting some of the downside effect of the buyout. I do not expect to see production this year below that of 1985, but in 1987 there will be a dip and within two years, with conditions as they are today, production will be back to the 1985 level and by 1990 production could exceed this year's level by some 11 billion pounds. This prediction presents more of a policy concern than any worry about an adequate milk supply.

Another reason that the buyout will not have as pronounced an effect on production as might first be assumed is the replacement aspect. Some 951,000 cows and 599,000 heifers and calves are being removed, but replacement heifers calculate out to only 38.8 per 100 milk cows compared to a national average of about 43 heifers per 100 milk cows.

The buyout is considered another experiment in hopes that the surplus will melt away. Probably one could say that the program is one step better than the diversion program to the extent that the participating animals will be slaughtered or exported. But as a solver of the surplus, the program will have only a temporary effect.

Presented by Hollis A. Hatfield, Director AFBF Dairy Division, at the 8th Biennial Cheese Industry Conference, Utah State University, August 26, 1986.
One of the oddities of the Food Security Act of 1985 was passage of the buyout, a decrease in the support price, and levying of a producer assessment to discourage milk production, but in the same breath the Act countered these actions by raising the Class I price in 35 federal order markets. Increases averaged 48 cents, ranging from seven cents to $1.03 per hundredweight.

I remarked at the time that the two ends were working against the middle and that the stage was set for some kind of retaliation. The Upper Midwest was not long in responding with a counter measure. Legislation has been introduced by two Wisconsin Congressmen that would merge the current 44 federal orders into one nationwide order and permit the pricing of reconstituted milk at other than the Class I price. The dairy industry never lacks for an issue.

Some Supply Factors

What is an adequate supply? Many producers are beginning to think that government purchases totaling 13 to 16 billion pounds per year are normal, but the track record prior to 1980 indicates otherwise. During the 31 year period 1949 to 1979, purchases averaged 4.9 billion pounds milk equivalent per year; since 1980, 13.2 billion pounds per year. A CCC purchase level of 5 billion pounds (milk equivalent) per year is a realistic goal.

The nation’s dairy herd totaled 11.1 million cows just prior to the herd buyout program. Allowing for a substantial increase in commercial sales, my estimates show that about 8.4 million cows will be adequate to supply the market in the year 2000. I have seen estimates as low as 7.8 million cows. But, again, in historical perspective, what is new? The projected decrease of 2.7 million cows by 2000 is a continuation of a trend that has been occurring for decades, but was derailed in 1980. And placed in perspective, the projected decrease would average 192,857 cows per year; small relative to the 1944-1979 period average annual decline of 425,714 cows.

Fewer cows means fewer dairymen. This is one reason why interest is being expressed for some type of a national supply control program. But I would hasten to add that preserving the status quo has not been a hallmark of quota programs. We need only to look at Canada’s experience. Between 1971 and 1985, the number of dairy farms in Canada decreased 63.7 percent compared to a decline in the United States of 56.5 percent and a drop in this country’s largest dairy state, Wisconsin, of only 32.3 percent.

Another significant change within the milk supply sector is the number of replacements being kept per 100 milk cows. Primarily because of a lower mortality rate and the continuing upgrading of the dairy herd, more heifers are being raised. From 1965 to 1969, an average of 31.1 heifers were kept per 100 milk cows; from 1970 to 1979, 34.5; and from 1980 to 1985, 42.5. Currently, about 43 heifers are being raised per 100 milk cows, an increase of 42 percent since 1965. It is this setting that has prompted an idea that removing heifers would be the most economical means of reducing the milk surplus.

If the current law is permitted to operate the duration of the 1985 Act, there could be a convergence of production and consumption sometime in the early 1990’s. The support price is scheduled to be reduced from $11.60 to $11.35 on January 1, 1987 and to $11.10 on October 1, 1987. The Secretary has the authority to reduce the support price to $10.60 per hundredweight for 1988, $10.10 for 1989 and $9.60 for 1990 if purchases are estimated to exceed 5
billion pounds (milk equivalent) in these calendar years. The impending price cuts are another reason for interest in quotas; the assumption being that most of the price cuts could be avoided if quotas were in place.

I would be naive to infer from this capsule analysis of the supply prospects that there will not be any areas of tight supply this summer, this fall, or in 1987, or at some future date. We have some areas of tight supply now; that is, tight supply for the commercial market. And all of you know why. It's this type of situation that has prompted the idea of a limit or cap on CCC purchases.

**Government Programs**

With this brief review of the supply situation, let's pursue the question... what turn might government programs take and what effect might these programs have on the milk supply?

A review of farm program history shows that whenever government surpluses have mounted to a relatively high level, mandatory controls have been advocated. This is the current setting. The excess production of many farm commodities, coupled with a downward pressure on price, is sparking a renewed interest among producers for mandatory controls. The current interest, however, differs substantially from some previous programs and proposals. The emphasis is on marketing quotas; not production quotas. A marketing quota relates to the bushels or pounds of a product marketed; a production quota, to acreage or number of animals.

Many dairymen are saying that none of the programs implemented in recent years including lowering the support price, imposing assessments, and authorizing a diversion program have brought the nation's milk supply into a reasonable balance with commercial sales. The herd buyout, as we have already noted, is considered a temporary measure. And probably not much relief can be expected from rising grain prices as a curb on milk output. It is in this climate of frustration that many producers are saying..."Let's try something new."

The voting delegates to the 1986 American Farm Bureau annual meeting stipulated that..."alternative programs for tailoring milk production to market requirements should be explored..." Material has been prepared on 11 proposals:

* A Voluntary Production Control Program
* All Class Base Proposal
* Two-Tier Supply Management Plan
* Self-Help Proposal
* Dairy Quota Proposal
* Freeman's Proposal
* Flexible Support Price
* CCC Purchase Limitation
* The Canadian Dairy Program
* Target Price-Deficiency Payment Program
* Marketing Contracts

These proposals encompass options that would take another approach to farm programs by altering or eliminating the current programs to marketing contracts between a cooperative and its members or between a handler and his shippers.

Farm Bureau's current position favors a flexible support price that is adjusted according to the amount of net dairy products purchased by the federal
government. A flexible support price is not new -- this was the type of program that operated effectively from 1949 to 1977, reduced excessive price variability, put a floor under producer prices, provided an adequate milk supply, and kept government purchases within a reasonable balance with market requirements.

The track record of the 1949 Act indicates that if the original concept of adjusting the support price, up or down, based on the nation's milk supply had continued in effect from 1977 to date, with the exception of the parity concept, the industry would not be faced with the unneeded buildup in cow numbers and the resultant downward pressures on producer prices.

At the other end of the dairy proposal spectrum are various mandatory quota proposals. The primary requisite of mandatory quotas involves placing an effective ceiling on the total quantity of the commodity that can be marketed. The programs being proposed are national in scope, applicable to all producers and all milk, and incorporate a price for quota marketings and a penalty for marketings in excess of quota.

A cursory review of the milk supply sector would suggest that there is justification for some change of attitude among producers. For example, the notion that a dairyman can produce whatever volume he desires and that someone must buy all that he produces should be dispelled. Such a stance is counter to market discipline; that is producing for a market. Agriculture, however, is not usually considered a disciplined industry. Such a stance conflicts with the desire for individual freedom. Discipline, however, is an integral part of success. Our actions. Our dress. Our habits. Our attitudes. Our pocketbooks. What is wrong with marketing contracts? What is wrong with producing for the market? And this state, Utah, has its hat in the idea arena with a voluntary production control proposal. The author correctly states that "a mandatory quota makes every dairyman become a participant by government edict."

Some producers are voicing a view that the current program encourages production because the program permits unlimited purchases by the CCC. Others argue that the most economical way to achieve the government's goal of reducing costs is to reduce purchases by the Gramm-Rudman percentage. The signal that such an option would give dairymen and handlers is that if you plan to increase production, then you must plan to move it into the market, not into the government store house.

Our Economic Research Division just completed an analysis of four dairy programs: Two-Tier Supply Management; Flexible Support Price; CCC Purchase Limitation; and Target Price-Deficiency Payments. The time period covered was 1988 through 1990. A brief summary of the analysis shows that the alternatives fall into two general groups, one is more market driven while the other is government set price driven. Present law, the flexible price support based on the manufacturing milk price, and the target price program lower the price support enough in 1988 through 1990 so that market forces play a role in setting the price of milk. The target price program, however, had the highest costs because of the deficiency payments.

The CCC purchase limitation, at the current support price, and the two-tier supply management set the price support at a high enough level that producers and consumers do not communicate through price and some type of output restrictions are necessary to balance production with consumption. These programs generally
have lower government costs. While this leads to higher incomes in the short run, the two-tier program in particular limits opportunities in the long run by limiting consumption.

The debate is just beginning. Although the Act of 1985 has four or more years to go, there is strong sentiment in some circles for a supply management program by October 1, 1987 when the buyout program terminates.

**Mandatory Controls and The Cheese Industry**

The topic of this session is directed to the supply side, but with the types of government programs being proposed, perhaps the key concern should be the market aspect of your business. All of the proposals focus on regulating the milk supply. Seldom is any question raised about a proposal's effect on the demand for dairy products, imitation products, or the competitive position with other foods.

With so much talk about quotas, what effect might a government supply control program have on the cheese industry?

First, let's look at the market for cheese. Cheese is the major bright spot in dairy product sales. Between 1983 and 1985, commercial milk use in the United States increased 8.5 billion pounds but nearly 60 percent of the increase can be credited to cheese. However, with all the talk about the gain in milk sales the role of cheese as a consumer of milk is often overlooked.

The cheese market is changing, the product mix is changing, but, in total, cheese sales have the potential to continue to expand and based on per capita use in some states, and in other countries, cheese has the greatest potential for sales gain of any dairy product. It was interesting to note that these comments were echoed in a cover story and feature article in the July issue of "Dairy Foods."

Now, back to the question, what effect might a mandatory supply control program have on the cheese industry? Our analysis, which I briefly alluded to, indicates that commercial sales would run less under a supply restriction program than under the current law. A study from the University of Wisconsin emphasizes that when milk quotas are used to try to elevate prices above the competitive levels, interregional equity becomes a serious concern. Consumption of fluid milk products is considerably less sensitive to price than consumption of manufactured milk products. An across-the-board farm level milk price increase would reduce manufactured product sales by as much as four times the percentage reduction in fluid sales.

Looking again at our neighbor to the north, theory suggests that if quotas restrict supply, prices could be pushed higher than they would otherwise be. Milk prices, however, must be reasonable in light of prices for other commodities, substitute products, and consumer incomes. One of the problems confronting Canadian dairymen is whether their supply management program has pushed milk prices out-of-line with other foods. Domestic use of milk products has been stagnant in recent years. In comparison, U.S. commercial dairy product sales increased 10 percent during the past six years.

The cheese industry has a vested interest in the dairy program debate. The well being of your industry's future could hinge on the type of dairy program that is implemented.
The dairy industry will continue to be confronted with adjustments as production and marketing changes arise. It probably will be well into the next century before there will be a market adequate to absorb the milk supply from a herd comparable to the number milked on January 1, 1986.

The business of dairying is not about to disappear. The current excess milk supply tends to obscure any positive aspects. Per capita sales for most products appear to have bottomed out and the increase in population is providing an expanding market base.

The central issue being shaped throughout the country is whether the milk supply should be regulated by a market driven program or by a government mandated control program. The cheese industry has more at stake in this issue than any other segment of the U.S. dairy industry.

Summary

The dairy industry will continue to be confronted with adjustments as production and marketing changes arise. It probably will be well into the next century before there will be a market adequate to absorb the milk supply from a herd comparable to the number milked on January 1, 1986.

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HAH:tlm
PRESENT AND FUTURE STATUS OF
WEST COAST CHEESE MARKETS

Dr. John W. Siebert
California Cooperative Creamery
P.O. Box 871, Petaluma, CA 95616

West Coast production of cheese has grown tremendously over the past fifteen years for two fundamental reasons. First, West Coast milk production has grown over this time period in response to favorable dairy farm economics. Second, the long term profitability prospects for manufacturing other dairy products from this flood of milk have less appeal than does the production of cheese.

Stated differently, there is nothing unique about the West's production of cheese. What is unique is the West's ability to produce milk.

As a consequence of Western expansion in the cheese industry, western cheddar block prices which once traded at a transportation premium over the National Cheese Exchange price now trade at this price level or even lower. How low can the price in the West go for this generic type of cheese? The answer is only as low as the National Cheese Exchange price minus the freight and handling for moving this Western cheese back to the Midwest.

For the future, this fundamental change in the locations of U.S. cheese production means that cheese pricing will be subject to less seasonal variation. Why? Because West Coast milk production patterns are less seasonal than those in the Midwest or the East. However, many people have yet to detect this trend because the USDA Milk Diversion Program masked it in the Fall and Winter of 1984-85 and because the USDA Milk Termination Program will mask it again in Fall and Winter of 1986-1987.

It is safe to predict that over time some of the large cheddar plants which have been built in the West will convert a portion of their production to Italian, Swiss, Mexican, muenster, edam and other more unique cheese varieties. The resulting increased supply of these varieties will in turn force their prices to bare a closer link to the National Cheese Exchange prices.

Future Growth

Many analysts have predicted that the cheese industry will continue to experience growth similar to the 3% to 10% annual growth rates which have occurred over the past fifteen years. Yet this prediction is based largely on the fact that such growth rates have occurred in the past. This fundamentally unsound
A method of economic projection is destined to fail sooner or later. This was certainly the case with the projections regarding the demand for beef, peaches, eggs and many other food commodities in the period of the mid-seventies versus the late seventies. Recently we have seen this fallacy of projection illustrated in the housing bust of the early 1980’s and the personal computer industry shake out of the mid-1980’s.

The earliest forewarning of this change in the cheese consumption trend will be a single future year in which consumption does not grow or in which it dips slightly. This will be followed by some small additional growth causing manufacturers to incorrectly rationalize that one dip in the trend means nothing. Yet the market for cheese must at some time turn static or downward and those building plants at that time will be left holding the bag for a costly and ill-timed investment.

But make no mistake about it, cheese has been the star sales performer of the dairy industry over the past fifteen years. Furthermore, when assessing the market for dairy products as a whole, I am totally optimistic. This is because milk is an amazingly fungible product. An example would be the new European style dairy products such as Totje, Flan, Fromage Frias and more which are being introduced here all the time. Pretty soon at least one of these will probably catch on big and boost the overall consumer demand for milk. Also, some basic items such as fluid milk, ice cream and sour cream continue to be strong performers due either to demographics (fluid milk and ice cream with the baby boom) or due to diet changes (the Mexican food trend with Sour Cream and the health trend with skim milk, nonfat yogurt and ice milk).

West Coast Marketing Trends

Several basic cheese marketing changes are occurring or about to occur in the West. Here are my predictions. First, we will see at least one very large cheese reprocessing facility built in the West. Currently trim and fines are sent to Mountain States or points east for processing and this will change as sufficient West coast production becomes available.

Second, the present level of California cheese production spells an end to the Western States’ importation of much of the cheese from Wisconsin and all other points east of the Mountain States. It is only a matter of time until Western cheese quality, variety, pricing, and marketing turn this prediction into a fact. You should in no way confuse this prediction with a prediction of high profits for Western cheese makers. Both Eastern and Western manufacturers will see slim profit margins and it is these slim profit margins which will force my prediction to become a fact. No one wants to fight a losing battle far from home.
Third, once the Federal Government gets milk production increases under control, you will see an end to the trend of new cheese plant construction in the West. Statistics published in the USDA Dairy Product Annual Summary, 1986 indicate that seven additional California cheese plants have been built over the past year.

Comment on Termination Program's Impact

The entire dairy industry is excited about the pricing boosting impact of the new USDA Dairy Termination Program. This program's effect will likely be very similar to the previous USDA Milk Diversion Program. One can thus predict the following. Cheese prices will remain above support from now until about May of 1987. After that time milk production will again be sufficient to return the cheese market to support.
CULTURES

AND

ENZYMES
A UNIQUE SYSTEM FOR ENHANCING 
CHEDDAR CHEESE AGING 

P. R. Freund 

Presented at the 7th Biennial Cheese Industry Conference 
Utah State University - Logan, Utah 
August 26-28, 1986 

Today's highly competitive cheese industry continues to intensify its search for new scientific technology to reduce product costs. Aged cheddar cheese requires considerable time to develop desired flavor and textural quality characteristics. This aging time adds significantly to product costs ranging from 1 1/2 to 3 cents per month per pound of cheese aged. Included are both the costs of providing refrigerated storage and the monetary investment covering the value of material held in inventory. Since some 2 billion pounds of cheddar type cheeses are manufactured annually in the United States, the development of an effective means for accelerating the aging process offers the potential of saving the industry millions of dollars annually.

After two years in development, a new and unique enzyme system for enhancing and accelerating cheddar cheese aging was introduced commercially in November 1985. This product, FLAVOR AGER-FR, was developed jointly by Genencor of South San Francisco, CA and Chr. Hansen's Laboratory of Milwaukee, WI. In 1982 Genencor, Inc. and Chr. Hansen's Laboratorium A/S, Copenhagen, Denmark entered into a joint venture agreement. Included in this agreement was the eventual development and marketing of calf rennet microbially produced as a result of recombinant DNA genetic engineering. In addition, other products for the dairy industry where the joint technical and marketing expertise of the two companies could be mutually beneficial were also to be included. FLAVOR AGER-FR is the first developed and marketed product of this joint venture.
In order to better understand the scope of this new development, we need to first explore what enzymatically occurs during natural aging of cheddar cheese and review affecting factors. (Slides #1-#7).

Over the years various methods, schemes and products have been developed to accelerate cheddar cheese ripening. Of the numerous attempts made, many have met with total failure, some with limited success under specific constraints, but none with total success. Before FLAVOR AGER FR development work was initiated, a list of developmental goals was agreed upon. (Slides #8 and #9).

The initial developmental breakthrough in the project was the discovery by Genencor scientists of a unique, new lipase enzyme obtained from a special single strain of Aspergillus oryzae. When this enzyme was added to dairy cream, incubated and allowed to react, a cheddar cheese-like aroma was found to occur. In addition, gas chromatographic free fatty acid analysis revealed that the resultant profile was very similar to that found in natural, aged cheddar cheese. Most reported and documented lipase enzymes have been found to either (1) preferentially release large quantities of butyric acid (C4) resulting in rancid off-flavors, or (2) preferentially release excessive amounts of longer chain free fatty acids (C12 and above) resulting in "soapy" off-flavors. This new lipase possesses neither defect.

Extensive fermentation, enzyme recovery and purification development work was necessary to supply sufficient quantities of this new lipase for cheddar cheesemaking trials.

Initial cheddar cheesemaking experiments with the lipase were conducted at the University of Kentucky in 400 lb. milk capacity pilot cheese vats under the direction of Professor C. L. Hicks. This work was reported by Frick et al. in 1984. These university studies also established that the use of a
specially selected protease, derived from a different strain of *A. oryzae*, in conjunction with the lipase yielded the best results. At this stage of development all enzymes tested had been mixed with the cheese salt and added to the cheese curds during the normal salting operation.

Commercial-scale production trials were first made with stirred-curd cheddar produced in 10,000 lb. open vats in a Wisconsin cheese plant in July 1984. Stirred curd cheddar was selected as this cheese is traditionally considered to require more storage time for producing aged cheddar flavor than that required with milled curd cheddar. Varied levels of enzymes with varied methods of application were evaluated. The laboratory/pilot plant procedure of adding the enzymes with the cheese salt was found to be totally unsatisfactory as non-uniform enzyme distribution which occurred resulted in inferior flavor development along with "enzyme hot spots" in the resultant cheese. The addition of the enzymes to the cheese milk 15 minutes prior to adding rennet was found to not only be the most convenient method but also the most effective.

Subsequent laboratory partitioning experiments demonstrated that when enzymes are added to the cheese milk 15 minutes prior to renneting, nearly all of the special lipase remained with the cheese curds. The explanation for the remarkably high recovery of the lipase was proven to be the result of this enzyme being in the form of water-insoluble microscopic size particles of an approximate diameter of 0.2 microns. These microscopic lipase particles disperse freely in the cheese milk, but are subsequently entrained during cheese curd formation. However, the added water soluble protease was found to partition as the cheese yields; namely, 10% stayed with the cheese curd and 90% went with the cheese whey.
Since 90% of the added protease was found to be passed into the cheese whey, extensive tests were conducted to determine the heat lability of the protease in cheese whey. It was determined that normal whey pasteurization conditions of 161-162°F for 15 to 16 seconds effectively inactivate the residual protease to undetectable levels.

Conducting of additional production scale trials was necessary to "zero in" on proper lipase and protease levels for acceleration and enhancement of long-hold cheddar cheese. This work culminated in the final development of FLAVOR AGER^FR. During this time stability storage testing of the packaged, hermetically sealed, enzymes was done. After storage for 1 year at room temperature (23°C) no loss in enzyme activities was detected.

Over the past 1 1/2 years commercial scale cheddar cheese plant trials have been made in each of the major milk shed regions of the United States including trials in Wisconsin, New York and California. In addition to the United States, commercial scale trials have also been made in Canada, Great Britain and Australia. Involved have been stirred curd and milled curd make procedures; pasteurized as well as "heat treated" milk; open as well as closed cheese vats ranging in size from 30,000 lbs. to 53,000 lbs.; cheese moistures ranging from 34% to 38%; cheese packing in 640 lb. blocks, 40 lb. blocks and 10 lb. blocks; and storage temperatures ranging from 40°F to 55°F. By the end of this month nearly 1/2 million pounds of cheddar cheese will have been successfully produced with FLAVOR AGER^FR. Two major U.S. cheese plants are presently producing FLAVOR AGER^FR treated cheddar on a routine basis.

In addition to subjective organoleptic evaluations, an objective method of analysis was needed to both "track" and quantify enzyme action in cheddar cheese during aging. Quantitative determination of free fatty acids (FFA) by gas chromatography was selected utilizing the method published by Deeth et al.
in 1983. Concentrations of the short chain free fatty acids (C4-C8) were found to effectively correlate with rates of cheddar cheese flavor development during aging. Additionally, individual free fatty acid profiles (C4 through C18:1) were found to correlate with balance in development of cheddar lipolytic flavor notes during aging.

Samples of retail purchased mild, medium, sharp, and extra-sharp Wisconsin cheddar cheeses were assayed for short chain FFA's. Ranges were determined and subsequently used for "baseline" levels in monitoring of commercial plant trials.

**Slide #10** presents short chain FFA data from two separate trials conducted in the same 50,000 lb. vat Wisconsin stirred-curd cheddar plant. The trials were conducted 5 months apart in time. In both trials the rate of short chain FFA release and flavor development was significantly greater with the treated cheeses than with the respective controls. In both trials, following initial accelerated lipolytic action, the rates of lipolysis in the treated cheeses were found to parallel that of the respective controls. The water-insoluble property of the special lipase was found to furnish a "shut-off" mechanism for accelerated lipolytic flavor development. Controlled experiments established that the discrete, dispersed, water-insoluble lipase enzyme in the cheese is only able to react with the milkfat molecules in the immediate vicinity of the lipase. When this action is completed, further lipolytic action in the treated cheese is derived only from natural sources. Thus, the lipase action is "substrate locale limiting".

**Slide #11** presents FFA profile data from the trials covered on the previous slide. Increased, but balanced, FFA levels are clearly shown for the treated cheeses.
Slide #12 demonstrates the similarity of FLAVOR AGER-FR performance in 38% and 36% moisture stirred curd cheddars.

Slide #13 presents FFA profile data for the cheeses covered in slide #12. Once again, increased and balanced FFA levels are demonstrated with the treated cheeses.

Slide #14 demonstrates the effects of block size on aging as measured by short chain FFA development. The positive effects of smaller block size as well as enzyme treatment are clearly evident.

Slide #15 presents FFA profile data for the same cheese covered on Slide #14.

Slide #16 presents short chain FFA data from a trial conducted in a 53,000 lb. vat Wisconsin milled curd cheddar plant. Similar results were found as shown earlier with stirred curd cheddar.

Slide #17 presents FFA profile data for the same cheeses covered on Slide #16.

Slide #18 presents short chain FFA data from two trials conducted with 33,000 lb. vat milled curd Wisconsin cheddar produced with heat treated rather than pasteurized milk. Again, the effect of the enzyme treatment is similar to that shown before.

Slide #19 presents FFA profile data for the same cheeses covered on Slide #18.

Slide #20 demonstrates the effect of storage temperature on short chain FFA's of FLAVOR AGER-FR treated cheeses.

SLIDE #21 presents FFA profile data for the same cheeses covered on Slide #20.
Slide #22 presents short chain FFA data from a commercial New York cheddar trial utilizing heat treated milk. Again, similar results were found to that previously shown with Wisconsin trials.

Slide #23 presents FFA profile data for the same cheeses covered on Slide #22.

Slide #24 presents short chain FFA data from a commercial trial conducted with stirred curd cheddar produced in California. Development of generally recognized sharp or extra-sharp flavor during aging of cheddar produced with California "valley milk" has not truly met with success over the years. It has been suggested that composition of the milk obtained in the central valley region of California is the primary cause. However, the exact nature of the difference between this milk and milk used elsewhere has never been fully established. The data presented on this slide and Slides #25 and #26 may offer some answers. No significant increases in short chain FFA released with aging are seen with the control cheeses stored at either 40°F or 45°F. Treated cheese stored at 40°F acted the same as the control, but the same cheese when stored at 45°F demonstrated significantly increased FFA release. Organoleptic evaluations of the 40°F stored cheeses demonstrated nearly identical, mild cheddar characteristics. The control cheese stored at 45°F demonstrated increased proteolytic flavor development, but the flavor was unbalanced due to lack of a corresponding lipolytic flavor development. However, the flavor of the treated cheese stored at 45°F had a balanced, aged cheddar flavor.

Slide #27 presents short chain FFA data from a trial conducted in Canada which once again parallels previous results.

Slide #28 presents FFA profile data for the same cheese covered on Slide #27.
Slide #29 presents soluble protein data derived from the Canadian study. The role of the protease in "speeding up" only the casein to peptide breakdown portion of the proteolytic enzyme scheme is quite evident. Differences in soluble protein levels between treated and control cheeses diminish after 2 and 3 months storage.

Slide #30 summarizes the role FLAVOR AGER-FR plays in the Enzyme Action Scheme of aged cheddar cheese.

In summary, the results of extensive commercial production trials have established that desired, enhanced and accelerated aging will occur when cheddar cheese is produced with this easy to use, unique enzyme system. It has also been established that the use of this system may also offer opportunities for production of quality, aged flavor cheddar cheese in plants where previously this was not feasible or economically practical.

References


Enzyme Action Scheme for Flavor and Textural Development of Aged Cheddar Cheese

- Casein: Protease from starter culture, milk, and coagulant
- Butterfat: Lipase from milk and starter culture
- Peptides: Peptidases, primarily from starter culture
- Free Fatty Acids

Typical Proteolytic Aged Cheddar Flavor and Textural Characteristics
Lipolytic-Proteolytic Derived Flavor Compounds
Free Fatty Acid Derived Cheddar Cheese Flavor Notes

Factors which can affect the quality of aged Cheddar cheese

I. The Milk Used

II. The Coagulant Used

III. The Cheese Starter Culture Used

IV. The Method of Cheddar Cheese Manufacture

V. How the Cheese is Stored
FACTORS WHICH CAN AFFECT THE QUALITY OF AGED CHEDDAR CHEESE

I
The Milk Used

A. Quality of the raw milk
B. Composition of the milk
  1) Protein/Fat ratio
  2) Minerals
  3) Miscellaneous
C. Whether milk used is pasteurized or heat treated

II
The Coagulant Used

A. Animal derived
   1) Calf rennet
   2) Bovine rennet
   3) Porcine pepsin
   4) Blends
B. Microbial derived
   1) Mucor miehei
   2) Mucor pusillus var. Lindt
   3) Endothia parasitica
   4) Recombinant DNA, genetically engineered microorganisms

III
The Cheese Starter Culture Used

A. The culture
   1) Type - bulk starter or direct vat set
   2) Nature
      a) Strains contained
      b) Purity of the culture
B. Bulk starter media used
C. Bulk starter system used
   1) Conventional
   2) Internal pH control
   3) External pH control
FACTORS WHICH CAN AFFECT THE QUALITY OF AGED CHEDDAR CHEESE

IV
The Method of Cheddar Cheese Manufacture

A. Type of Cheddar made - stirred curd vs. milled curd
B. Target moisture content of cheese
C. How the cheese is packed
   1) 10 lbs. and smaller
   2) 40 lb. blocks
   3) 640 lb. blocks and larger
   4) Barrel

FACTORS WHICH CAN AFFECT THE QUALITY OF AGED CHEDDAR CHEESE

V
How the Cheese is Stored

A. Form of cheese placed in storage
B. Temperature of storage
   1) 32°-36° F
   2) 40°-48° F
   3) 50°-54° F
   4) Combinations of the above
C. Time of storage

<table>
<thead>
<tr>
<th>Cheddar Type</th>
<th>Typical Age</th>
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<tbody>
<tr>
<td>Mild</td>
<td>3 months</td>
</tr>
<tr>
<td>Medium</td>
<td>6 months</td>
</tr>
<tr>
<td>Sharp</td>
<td>9 months</td>
</tr>
<tr>
<td>Extra sharp</td>
<td>12 months or more</td>
</tr>
</tbody>
</table>
GOALS SET FOR DEVELOPMENT OF A
PRODUCT TO ACCELERATE THE RATE OF
RIPENING OF CHEDDAR CHEESE

1. The use of the product yields clean, balanced proteolytic and lipolytic flavor notes throughout the aging process, but at an accelerated rate.

2. The product is simple to use and production application does not require alteration of presently used cheese making processes.

3. The product is adaptable to different cheddar cheese make procedures.

4. The product reacts uniformly within a vat of cheese as well as from one cheese vat to another.

5. The accelerated action of the product is controllable.

6. The product requires no special handling prior to use, and is preferably shelf-stable at room temperature (23° C).

7. The product has no adverse affect on the utilization of cheese whey derived.

8. The product is economically cost effective to use.
Wisconsin Stirred Curd Cheddar

640 Pound Blocks - 35% Moisture - 40 F.

Weeks After Manufacture

Control 1  +  Flavor Age 1  ○  Control 2  △  Flavor Age 2

Wisconsin Stirred Curd Cheddar

640 Lb. Blocks 40 F.

Specific Free Fatty Acid

Control 2 20 Week  ×× Flavor Age 2 20 Week  ▣ Control 1 40 Week  ×× Flavor Age 1 40 Week
Wisconsin Milled Curd Pasteurized Milk

640 Lb. Blocks - 41 F.

PPM Short Chain FFA (C4-C8)

Weeks After Manufacture

- Control
+ Flavor Age - FR

Extra Sharp
Sharp
Medium

Wisconsin Milled Curd Pasteurized Milk

640 Lb. Blocks - 41 F. - 28 Weeks Storage

PPM FFA Released (Thousands)

Specific Free Fatty Acid

- Control
- Flavor Age - FR
Wisconsin Milled Curd Cheddar

Heat Treated Milk - 640 Lb. Blocks

PPM Short Chain FFA (C4-C8)

Weeks After Manufacture

- "K" Control
- "K" Average
- "W" Average

Flavor Age - FR

Wisconsin Milled Curd Cheddar 26 Week

Heat Treated Milk - 640 Lb. Blocks

PPM FFA Released

Specific Free Fatty Acid

- "K" Control
- "K" Average
- "W" Average

Flavor Age - FR
Milled Curd Wisconsin Cheddar

Temperature Study

Weeks After Manufacture

PPM Short Chain FFA (C4-C8)

Control 1  +  Flavor Age FR  ○  Flavor Age FR  △  Flavor Age FR  ×  Flavor Age FR  ▽  Control 2
40 F.  Val 1 40 F.  Val 1 55 F.  Val 2 40 F.  Val 2 55 F.

Milled Curd Wisconsin Cheddar 26 Week

Temperature Study - FFA Profile

Specific Free Fatty Acid

Control 40 F.  Flavor Age FR Val 1 40 F.  Flavor Age FR Val 1 55 F.  Flavor Age FR Val 2 40 F.  Flavor Age FR Val 2 55 F.
California Stirred Curd Cheddar
40 Lb. Blocks Stored at CHL Milwaukee

FFA C4-C6 (PPM)

0 10 20 30 40
Weeks After Manufacture

- Flavor Age • Flavor Age ○ Control △ Control
Vat 10 Vat 10 Vat 13 Vat 13
40 F. 45 F. 40 F. 45 F.

California Stirred Curd Cheddar
40 Lb. Blocks Stored at CHL Milwaukee

PPM C10-C18 FFA (Thousands)

0 0.5 1 1.5 2 2.5 3 3.5 4
0 10 20 30 40
Weeks After Manufacture

- Flavor Age • Flavor Age ○ Control △ Control
Vat 10 Vat 10 Vat 13 Vat 13
40 F. 45 F. 40 F. 45 F.
Free Fatty Acid Profile
California Stirred Curd Cheddar 40 Lb. Blocks Stored 36 Weeks At CHL Milwaukee

- **Specific Free Fatty Acid**
  - Control Vol 13 40 F.
  - Flavor Age Vol 10 45 F.
  - Flavor Age Vol 10 45 F.

Canadian Milled Curd Pasteurized Milk
40 Lb. Blocks 5 C.

- **Extra Sharp**
- **Sharp**
- **Mellow**
Canadian Milled Curd Pasteurized Milk

40 Lb. Blocks 5 C. 13 Weeks Storage

Specific Free Fatty Acid
- Control
- Flavor Age

Soluble Protein Profile 40 Lb Block

Milled Curd Canadian Cheddar Stored at 5 C.
Enzyme Action Scheme for Flavor and Textural Development of Aged Cheddar Cheese

Casein protease from starter culture, milk, and coagulant

Butterfat lipase from milk and starter culture

"Envelopes" around protein to allow subsequent proteolytic action

Free Fatty Acids

Peptides, primarily from starter culture

Typical Proteolytic Aged Cheddar Flavor and Textural Characteristics

Lipolytic-Proteolytic Derived Flavor Compounds

Free Fatty Acid Derived Cheddar Cheese Flavor Notes

FLAVOR AGE™
During the past twenty years, the United States has experienced an increasing demand for cheese and cheese-products. The rural cheesemaking scene of numerous, small, family-run operations has largely yielded to consolidation to form a few, very large cooperatives. Joe O'Donnell, of the Dairy Research Foundation, speaking at the June ADSA meeting stated that "from 1975 to 1984 the number of cheese plants dropped by 20 percent, whereas output increased by 105 percent." Cooperatives, processing millions of pounds of milk per day into cheese, have, out of economic necessity and increasing milk-volumes, moved to highly-mechanized systems, and have adopted new technologies and improvements as they appear.

Ten years ago, at the Second Biennial Cheese Industry Conference, a report (1) was given outlining the first-years's experience of growing bulk starter under pH-control conditions; a concept pioneered here at Utah State University. Over the ensuing decade this technology has weathered the test of time and has now become an industry standard for growing bulk starter. I would venture that well over 50% of North America's Cheddar and barrel cheeses are manufactured with some form of pH-controlled starter.

But what is pH-controlled starter, and what advances have been made over the past decade in this technology? As starter bacteria ferment lactose to lactic acid, the buffering-capacity of the growth medium is eventually overcome and the pH drops until acidic conditions become unfavorable for the bacteria and acid-production stops; usually around pH 4.6-4.7 (Figure 1). According to Harvey (2), starter-growth in an environment below pH 5.0 results in reduced enzyme activities.

If starter bacteria remain at this low pH for extended periods of time, a steady decrease in acid-producing activity results and increasing amounts of starter must be used to form comparable amounts of lactic acid over a given period of time. Cheese makers refer to this condition as "over-ripened starter." This condition can be reversed by transferring and growing the starter at pH values above 5.0. Conventionally-grown starter (no pH-control used) is usually added to cheese milk as the vat is filling, to allow a 20 to 50 minute "ripening" period. During ripening, starter

Fig. 1 Typical growth curve of mesophilic lactic acid bacteria.
bacteria are able to repair and overcome their "acid-induced" lag phase and move into log phase, with its rapid cell growth and acid production.

Figure 2 compares starter growth curves under external and internal pH control with starter grown in conventional phosphated-media. Under external pH control conditions, pH is maintained above the point where acid-injury to the starter bacteria occurs. This is done either automatically through instrumentation, or by manual one-step or multiple-step addition of neutralizer. When fitted with instrumentation, the entire fermentation cycle is controlled automatically. An electrode mounted in the bottom of the bulk tank constantly monitors the growing culture, and when lactic acid build-up lowers the pH to a set-point (usually around 5.8) the controller activates an ammonia pump to neutralize the acid, and raises the pH to 6.0. As more acid is produced, the cycle is repeated. These neutralization-cycles are recorded as sawteeth

**Figure 3**

A schematic of pH and temperature control systems for a starter tank.
on a chart recorder. When lactose in the medium is depleted, the neutralization cycles cease, as seen by a straight-line on the chart. At this point the starter is cooled, and is ready to use.

"One-step" neutralization (3) consists of allowing the pH of the developing starter to drop to around pH 5.0, at which point the starter is neutralized to pH 7.0 with a single dosing of neutralizer, such as sodium or potassium hydroxide. Starter growth is allowed to continue an additional 2 hours, before cooling, to double viable cell counts.

Under internal pH-control (4), pH is maintained close to 5.2 by buffer salts which are insoluble at higher pH values. As lactic acid from a growing culture lowers pH, the buffer salts are gradually, and continually, solubilized to neutralize the acid. Patents on this technology were issued in the early 1980's. Starter activities for cultures grown under external and internal pH-control are somewhat comparable, and cell numbers are approximately ten times higher per unit volume, than under conventional conditions. Where starter has been maintained at a more optimum pH, and has not undergone acid damage, more active starter culture results. Hence, it is not uncommon for inoculum rates to be reduced by half over starter grown in traditional phosphated-media.

When pH-controlled systems were first introduced in the mid 1970's, the main selling point was economics (5). The cheapest available nutrient source was freshly-drawn whey, to which was added a supplement of yeast extracts, hydrolysates and phosphates. Table 1 compares today's cost of setting ten-thousand pounds of cheese milk with various types of starter media. The first four media were specially formulated for use under external pH control conditions. These media usually contain lower solids and are lactose-limiting. According to Ausvanodom et al (6), less phosphates are needed in external pH-control media to impart a specific degree of phage protection because pH is maintained around 6.0, where calcium is less soluble. In contrast, calcium becomes increasingly soluble as pH approaches 5.0, and greater amounts of phosphates are required to chelate soluble-calcium at lower pH values. Because of reduced phosphate levels, it is not necessary to phosphate-adapt starter cultures.

Although the economics of using fresh whey plus a nutrient supplement made pH control very popular, the latter part of the decade has seen a steady movement towards complete media, both whey- and milk-based. The reasons for this seem to hinge around
convenience and better starter consistency. The fresh-whey approach requires whey to be drawn off and separated, then pumped to the starter tank. A powdered supplement is then added and the lactose level is adjusted by diluting the whey with water.

Most inconsistencies in fresh-whey starter come from poor treatment and handling of the whey, improper whey dilution, and carry-over of bacteriocins or other inhibitors in the whey. The slide shows the effects of diplococcin, a bacteriocin given off by one starter strain that inhibits growth of a second, susceptible starter strain. We have seen limited cases where bacteriocin carry-over in whey has severely inhibited starter growth in the bulk tank. Complete media, on the other hand, are formulated with pre-tested ingredients to ensure high quality and consistency. A second factor is the potential yield from starter solids. There is some question as to what percentage of milk-based starter-solids can be incorporated into cheese curd. Values reported in the literature (7,8,9) vary from 0 to 40 to 96%. Further research in this area is on-going.

The most significant advances of the past decade in pH control, have occurred in the area of instrumentation. The original analog controller described by Richardson and Pearce (10), which turned a single relay on and off, has evolved through several generations during this past decade. The latest microprocessor-controlled instrument is capable of controlling two starter tanks simultaneously, yet independently, through pasteurization, cooling, multiple growth phases, and final cooling and holding.

Figure 4 outlines the past decade's evolutionary process in instrumentation. The original Great Lakes Instruments Controller had only a single ON/OFF relay which was used to open and close an ammonia gas valve leading to the bulk tank. The gas valve was soon replaced with a diaphragm pump, and later with a peristaltic pump attached to a barrel or tank of ammonium hydroxide. This model controlled only pH. A separate temperature controller had to be wired to a cooling solenoid. Second-generation instruments moved to microprocessor- and digital-control, which opened up a whole new world of parameter control. Temperature was now controlled by the same instrument, and temperature compensation was linked to pH measurement. Duty cycles for agitation and neutralization.

---

**Fig. 4 Evolution of pH-control instrumentation.**

- **Analog**
  - Single ON/OFF Relay
  - Separate Temp. Controller

- **Digital Control**
  - 4 Relays
    - Temperature (Cool) Control
    - Duty Cycles
    - Temperature/PH Compensation
    - Auto-Cooling

- **Switching Box for 2 Bulk Tanks**

- **Multiple Tank Control**
  - Heating & Cooling Control
  - Pasteurization/ Cool Control
  - Multi-Stage Cooling

- **Starter Processing Recipes**
  - Multi-Stage pH Control
  - Display Prompts
  - Custom Programming Available
were added. The number of ammonia injections could be specified prior to autocooling, and pH and temperature were recorded on the same chart. It should be emphasized that up to this point an instrument could control only one bulk tank, each tank requiring its own separate controller. This description is generally representative of the pH-control instruments distributed by the major suppliers. The next evolution produced a manual switching unit which allowed a single instrument to control two tanks alternately, but not simultaneously.

The latest evolution has brought forth an instrument capable of controlling two starter tanks simultaneously, yet independently of one another. Up to 10 automatic-processing recipes can be programmed into the instrument to control the entire starter-making process from pasteurization, through growth, to autocool. The instrument is also capable of multiple-stage pH control. This means that pH can be controlled in a certain range for either a specified number of ammonia injections, or a specified period of time, before moving to another pH-control range. This is particularly useful in adjusting rod-coccus ratios for various Italian-cheese starters. The instrument is capable of initially cooling the finished starter with well water, before switching to sweet water to complete the cooling process. Up to 16 different relays can be controlled at once. The instrument uses a Taylor or Partlow Recorder, and is priced under $10,000. With the rapid advances in microprocessor-technology, it is difficult to speculate what next-decade instruments will control; perhaps continuous starter-production linked to continuous cheesemaking?

Experience with the first pH-control system installed in a cheese plant (1,11), showed that the majority of mechanical problems with pH-control equipment stemmed from the short and erratic life of the pH electrode. The first probe had a salt bridge, and was mounted through the dome of the starter tank. Today's flowing-junction pH probe is mounted in the bottom of the starter tank, and is fitted with a pressurized immersion-assembly to overcome the head-pressure of the starter in the tank. The original Great Lakes Instruments probe, normally used in waste-treatment processes, was not designed to withstand the numerous high-temperature, extended-time, vat-pasteurization treatments given to bulk starter. This problem was corrected by switching to the Ingold pH electrode. This glass electrode can withstand the rigors of vat pasteurization or sterilization, as well as acid and caustic CIP treatments. With proper care and maintenance the Ingold electrode should have a normal life of up to a year. The most common problems we find with pH-control equipment still stem from poor care and maintenance of the pH electrode. Several cleaning, and reactivating solutions are provided to ensure optimum electrode performance. New electrodes, which claim longer life and greater reproducibility, are now beginning to appear on the market to compete with the Ingold electrodes.

Early pH-control systems used anhydrous ammonia as a neutralizer (12,13), which required an ammonia manifold, a feed tube which was submerged in the starter medium, and a vacuum-breaker check-valve
to prevent suction of media into the feed line. The feed tube had to be removed and hand-cleaned for each tank of starter, because of it's potential for harboring organisms and media. I am presently aware of only a handful of cheese plants in the U.S. that still use anhydrous ammonia. All of the other plants, which number well over two hundred now use ammonium hydroxide. Neutralizer is delivered through an over-head line, by a peristaltic pump, and is allowed to free-fall into the starter medium. The feeder line extends only about an inch through a ferrule-cap in the top of the tank, and can be easily cleaned in place. Because it does not come into direct contact with the medium, there is no place to harbor organisms or media.

One of the initial concerns at the introduction of pH control, a decade ago, was the potential for pathogen growth in the neutralized medium. Studies (14,15,16) have shown that when grown alone in pH-controlled media, growth of B. subtilis, E. coli, Salmonella, and to a slight extent S. aureus could be supported. However, in the presence of lactic starter organisms, negative growth curves were seen for all of the organisms, with the exception of E. coli, which could compete fairly well with the lactics. It should be noted that coliforms can continue to grow in finished starter, even though lactose is depleted. They are apparently able to use alternate energy sources for growth.

It is therefore, especially important that good manufacturing procedures (GMP) be used in inoculating bulk tanks, and in cleaning and sanitizing transfer lines and holding tanks to prevent coliform contamination. Luckily, pH-control recordings give us some limited monitoring capabilities for coliforms growing in the bulk tank. As lactose is depleted by the growing starter bacteria, acid production stops. This is indicated by a straight line on the recording chart (Figure 5). Because coliforms will grow, even in lactose-depleted medium, they continue to slowly produce acid which usually shows up as extended sawteeth on the recording chart after normal lactic fermentation has "straight-lined" or ceased.

Figure 6 shows data from our lab, comparing the activities of a starter culture grown under pH-control using three different neutralizers. These findings are well supported in the literature. Ammonium hydroxide has become the neutralizer of choice, primarily because of low cost and availability. Potassium hydroxide, and sodium hydroxide solutions are currently being used in one-step manual neutralizations, where cation concentrations do not become inhibitory (3). Sodium hydroxide cannot be used under
continuous pH-control conditions. All three neutralizers are listed under GRAS (Generally Regarded As Safe) status in the latest edition of the Food Chemical Codex (17).

The appearance of new technology usually brings with it its own set of unique problems and circumstances. At a recent professional meeting, a colleague voiced the opinion that, "The quality of American cheese has gone downhill. Cheese is being made too fast. pH-control has facilitated shortening the make-time."

There is no question that recent years have seen make-schedules for Cheddar cheese decrease significantly from 5 hours, down to present-day's 3.5 hours, or less. Processing today's huge milk-volumes requires faster manufacturing schedules, and round-the-clock production, as cheese making has moved from an artisan craft, requiring time and patience, to a time-regimented business. Milk microflora has changed also. Milk left out on the counter no longer sours, it rots!

But what about the role of pH-control in cheese quality? pH control has indeed helped shorten the cheese-making process by providing the cheese industry with a low-cost, consistent, and extremely active starter. Over the past decade, millions of pounds of high quality cheese have been made with pH control systems. But, these systems have also thrown the industry a manufacturing curve-ball. The use of traditional cheese-making methods, geared to over-ripened starter, has often resulted in acid/bitter cheeses when used with pH-controlled starter. Proper adjustments must be made in the make-process, to limit acid development in the beginning stages of cheese making. It is important to recognize the control points of this starter technology. pH-controlled starter contains approximately ten times more cells than traditional starter. These cells are healthy, non acid-injured, and are poised for action when they are added to cheese milk. Hence, lower inoculum rates must be used, and ripening periods must be drastically shortened or eliminated altogether. (Remember, ripening periods are used to overcome acid-injury to the starter).

Growth under pH-control conditions has also given extra "drive" to starter cultures i.e. cheese-pH continues to drop significantly after salting. Because of this phenomenon, high milling-acids 0.50 to 0.60 have been replaced by lower titratable acids of 0.40 to 0.47 %. Resulting cheeses usually have a 24-hour pH-value of 5.05 to 5.15.
The manufacture of high-quality cheese, under any starter system, requires the knowledge and careful control of all cheese-making parameters. The mixing of old and new technologies does not always work smoothly. As we adopt new technologies, we as an industry have the responsibility to learn and understand correct applications and controls of the technology.

The past decade has also witnessed the introduction of defined-strain technology to the U.S. cheese industry. The success of this culture-technology in the U.S. is largely based on its marriage to pH control systems. The increased activity and cell numbers, obtained through pH control, allowed the selection of medium-fast acid-producing strains which exhibit greater phage resistance. Because of increased phage resistance and greater starter activity, a single tank of starter culture can be prepared and used continuously in cheese making over several days. It is correct to say that U.S. defined-strain technology was designed specifically around pH control. Both technologies have served to enhance the benefits of the other.

While external pH-control using instrumentation, may not be suitable for all cheese plant environments, alternate methods of pH control may certainly be suitable. At the end of this first decade of pH-control, I believe that it can be accurately said that pH-control systems have weathered the test of time, and have helped shift cheese making from an art to a science. Thank you.

References


Use of genetic engineering principles to develop improved lactic starter culture strains has great potential to aid the dairy starter industry and cheesemakers. Since the 1930's, several starter-associated defects and problems have been observed. These problems include variability in important starter-associated metabolic activities such as lactose utilization and proteinase activity. Starter cultures defective in these metabolic activities result in "slow" acid production. In addition, variability in the ability of some strains to utilize milk citrate has affected aroma and flavor production. Many strains have produced undesirable off-flavors such as bitterness, and most strains are susceptible to bacteriophages. These factors along with increased cheese production schedules and the appearance of large mechanized plants have placed increased stress on existing starter strains. Because of these factors, the number of suitable strains for use in cheesemaking is rather limited and there is a continuing need to obtain new and improved strains that can perform efficiently and predictably for extended times.

Several strategies have been used to increase the number and efficiency of starter strains. Attempts have been made to isolate new strains from the natural habitat e.g. green plant material and milk, but this approach has been largely unsuccessful since most of these strains are unsuitable for cheesemaking. Mutagenesis of pre-existing starter strains followed by selection has been useful for developing bacteriophage-resistant mutants. However, these mutants may revert to phage sensitivity or may, after a short time, become susceptible to new phages. Two new strategies involve genetic manipulations. One genetic strategy is to use gene transfer systems to move desirable genes from one strain to another. The other strategy is to use recombinant DNA technology to alter specific metabolic capabilities. The genetic strategies have great potential since improvement of strains may be specifically directed, monitored, and controlled.

Use of genetic engineering principles relies on our knowledge of the genetics and microbiology of starter cultures. The most studied group of starter bacteria are the group N streptococci. This group includes strains of Streptococcus lactis, Streptococcus cremoris, and Streptococcus lactis subsp. diacetylactis. Genetic studies of the group N streptococci which were pioneered by Dr. Larry McKay at the University of Minnesota, have shown that plasmid DNA codes for many important properties which are required for or are associated with dairy
Plasmids are extrachromosomal, autonomously replicating, circular DNA molecules, which exist independently of the main genetic machinery of the cell, the chromosomal DNA. They are found in most bacterial species and under normal circumstances a particular plasmid is dispensable. However, plasmids may contain genes that are essential or confer a selective advantage to cells in certain environments. Plasmids are characteristically unstable entities and may be lost with a concomitant loss of particular traits coded for by that plasmid. This loss of plasmid DNA is referred to as plasmid curing.

It is now well established that the group N streptococci harbor a large complement of plasmid DNA. Some strains may contain up to fourteen distinct plasmids ranging in mass from one to 88 Mdal. On the average, most strains contain four to seven plasmids. Most of the plasmids are cryptic, i.e., there is no known function associated with a particular plasmid.

Properties coded for by plasmids which are important to dairy fermentations include lactose and galactose metabolism, proteinase activity, citrate utilization by *S. diacetylactis*, production of antagonistic properties (bacteriocins, nisin, and diplococcin), and several mechanisms of resistance to bacteriophages. Therefore, plasmids studies in group N streptococci have been clearly important for many reasons. First, it is known that plasmids may be lost which explains the irreversible loss of properties required for successful dairy fermentations, e.g. lactose metabolism, proteinase activity, citrate utilization, and phage resistance. Bacterial cells may also acquire plasmids via gene transfer mechanisms thus acquiring new properties. These gene transfer systems will be discussed later and have important implications for improving strains used in milk fermentations. Plasmid studies also have a vital role in developing recombinant DNA technology, a most powerful tool for improving strains used in fermentation.

Transfer of genetic information among group N streptococcal strains occurs by at least four distinct mechanisms: transduction, conjugation, protoplast fusion, and protoplast transformation. These gene transfer mechanisms are essential for genetic studies and are vital for strain construction strategies to improve starters.

Transduction is a gene transfer mechanism by which a bacteriophage is an intermediate in the transfer of genes from one strain to another. This occurs when a strain is infected by a bacteriophage. During the normal course of bacteriophage replication, host bacterial genes may be accidentally packaged into the phage head instead of phage genes. After production of phage particles within a host, phages are released by lysis of the host. The released phages then infect other cells and transfer the genetic information to new hosts. In the group N streptococci, several traits have been transduced including plasmid-coded lactose metabolism and proteinase activity.

A second method of gene transfer is termed conjugation. Conjugation requires a donor and a recipient cell to come into physical contact whereby the donor strain will transfer genetic
information to the recipient strain. This gene transfer mechanism has been extensively used in the group N streptococci for genetic analysis and for strain improvement.

The next two methods of gene transfer require the formation of bacterial protoplasts. Protoplasts are cells with the cell wall enzymatically removed, leaving the cell surrounded by the cell membrane. Protoplasts are osmotically labile, but by maintaining them under hypertonic osmotic conditions, usually in the presence of high sugar or amino acid concentrations, they remain viable and do not show osmotic lysis. Regeneration or resynthesis of cell walls and subsequent growth into bacterial colonies may occur on solid media containing an osmotic stabilizer. Protoplasts are currently being used for transferring genes through protoplast fusion and protoplast transformation.

Protoplast fusion involves the formation of protoplasts from two phenotypically distinct strains. These two populations of protoplasts are mixed together and then induced to fuse by the addition of the fusogenic agent, polyethylene glycol or PEG. When two cells have fused together, genetic information is recombined so that the resultant fused cell now contains genetic information from both strains.

The last mechanism of gene transfer is termed protoplast transformation. Transformation is the uptake of free or naked DNA by a cell resulting in the acquisition of new genes. Uptake of DNA by protoplasts is facilitated by treatment of protoplasts with DNA and PEG. Once protoplasts have taken up DNA, they are plated on media containing an osmotic stabilizer where they regenerate their cell walls and grow into visible transformant colonies. Transformation, as we will see later, will be a vital gene transfer system for studying the genetics of the group N streptococci, but more importantly, it is a required procedure for the use of recombinant DNA technology for strain improvement.

Recent studies on plasmids in group N streptococci and the development of gene transfer systems have facilitated the development of recombinant DNA or gene cloning techniques. Recombinant DNA techniques rely on our ability to cleave DNA molecules with restriction endonucleases and to join DNA molecules to form hybrid or recombinant DNA molecules. Restriction endonucleases are enzymes which are able to cleave DNA molecules in specific places. There are over 400 different restriction endonucleases which are able to cleave DNA with different specificities.

To clone a gene, a DNA carrier molecule termed a cloning vector and DNA with a desired gene to be cloned (passenger DNA) are required. The cloning vector is usually a plasmid which is able to replicate in appropriate recipient cells. It must have a readily selectable phenotypic marker and a unique restriction endonuclease site in which to insert passenger DNA. In a typical cloning experiment, vector and passenger DNA are usually cleaved with the same restriction endonuclease, producing linear vector molecules and several passenger DNA fragments with "sticky" ends. When vector and passenger DNA are mixed, random insertion of passenger fragments into the vector forms hybrid or recombinant
DNA molecules. This occurs due to base pairing of "sticky" ends. Hybrid molecules are covalently sealed using the enzyme DNA ligase. Recombinant DNA molecules are then transformed into appropriate recipient cells where many copies of the recombinant DNA's are made. When this occurs, the inserted passenger DNA fragment is said to be cloned. Transformant colonies are then screened to determine if the desired gene was cloned.

The recombinant DNA techniques described have been used to clone genes in group N streptococci. However, all experiments to date have used plasmid cloning vectors which contain potentially harmful genes e.g. antibiotic resistance genes, from other microorganisms such as Streptococcus sanguis, Bacillus subtilis, and Escherichia coli. Exploitation of the potential that recombinant DNA techniques has to offer will require the development of a food grade cloning vector. This vector should only contain DNA and genes from food grade microorganisms such as the group N streptococci. In addition, the vector should not contain antibiotic resistance genes for markers since they may be potentially transferred to pathogenic intestinal microflora. Until a food grade cloning vector is constructed, the development of improved strains by recombinant DNA technology is still in the near future. Soon after the development of a food grade vector, improved strains constructed using recombinant DNA techniques should soon be available for field trials and eventual use by the dairy fermentation industry.

Several laboratories worldwide have been researching the genetics of group N streptococci with potential applications in mind. The most important applications of genetic technology include the development of starter strains with enhanced bacteriophage resistance, accelerated cheese ripening and flavor producing capabilities, agglutinin resistance, and production of inhibitory microbial agents such as nisin for food preservation. Research has also aimed at the stabilization of plasmids by their integration into a stable chromosomonal location. Research which does not concern starter cultures but which has important implications for the dairy industry include cloning the calf rennet genes in bacteria so the enzymes may be produced by fermentation techniques rather than being extracted from calf stomachs and the production of casein by microorganisms in a bacterial fermentation process.

Major breakthroughs in providing improved strains using genetic technology have recently occurred. The first, was in the development of strains with increased bacteriophage resistance properties. Dr. T. R. Klaenhammer at North Carolina State University in collaboration with Miles Laboratories have studied plasmid-linked mechanisms of bacteriophage resistance. They have been able to transfer a plasmid coding for a variety of mechanisms of phage resistance to a cheesemaking strain which was unable to be used because of it's phage sensitivity. When the cheesemaking strain acquired this new plasmid via conjugation, it became resistant to plant phages. In pilot studies and in field trials, this strain has remained phage resistant in 6 months of plant use. These results demonstrate that a good cheesemaking strain which could not be used because of its phage sensitivity,
could be become phage resistant by transfer of a single plasmid through the gene transfer technique, conjugation.

Another breakthrough which has used genetic technology was performed at Oregon State University by Dr. W. E. Sandine. Sandine's group was able to transfer the nisin production and resistance genes as well as the lactose utilization genes from \textit{S. lactis} to \textit{Leuconostoc} via conjugation. Nisin is a bacterial inhibitor produced by \textit{S. lactis} which is used in Europe as a food preservative and should be approved, in the near future, for use in the United States. This compound was shown to be overproduced and excreted in \textit{Leuconostoc}. Therefore, production of nisin for food preservation in \textit{Leuconostoc} would be much more profitable than producing it in \textit{S. lactis}. In addition, by transfer of the lactose plasmid from \textit{S. lactis} to \textit{Leuconostoc}, transconjugants could now utilize lactose in milk to produce acid. This newly acquired trait thus allows \textit{Leuconostoc} to produce the flavor compound, diacetyl, alone rather than relying on associative growth with an acid producer to produce diacetyl.

In other studies, McKay's research group at the University of Minnesota have isolated temperate sensitive mutants which lyse at cooking temperatures. Although pilot studies have yet to be performed, use of these mutants should be able to accelerate the cheese ripening process.

These significant results were acquired through our knowledge of the genetics and microbiology of lactic acid bacterial starter cultures. As an important note, these results were obtained through simple gene transfer systems. The potential of recombinant DNA techniques is yet to be realized. Further research and development is needed as well as cooperation between dairy technologists and molecular biologists to fully exploit the potential genetic engineering has to offer.
REFERENCES


References for this presentation are not listed throughout the text but were taken from the above review articles. Recent results were obtained through personal communications with Drs. W.E. Sandine, L.L. McKay, and T.R. Klaenhammer.
This late in the day there are two reasons why I should stick with Tony Ernstrom's suggestion to just tell jokes. First, the steak fry is fast approaching. Second, we have become accustomed to declaring all cheese yield claims as just plain unbelievable. I recall what a tough time I gave Bob Sellars when he first showed me data demonstrating that direct-to-vat cultures could improve cheese yield. I know now why they do, but the logic escaped me then. Try and explain how you measure yields and watch for the reactions, "You can't use total solids because sodium frustration is not a cheese solid", "You can't report wet weight", "That is an indirect measure and does not work in the real world", "That is fine in the ivory tower, but have you tried it in a plant?" You have certainly heard statements that it is impossible to measure cheese yields in large plants, that measurements made in pilot plants are inaccurate and that those made in laboratories are impossible. My response has been, "Then what is required before it becomes believable?" The replies go in many directions, but still boil down to plant, pilot plant or laboratory studies, depending upon who is responding. I shall try to include examples that have been gathered using many approaches to update you on yield claims where lactic cultures are involved.


Proteolysis vs. Yields.  2

Today Jeff Kondo described the great interest in learning more of the genetic characteristics of the lactic culture bacteria used in cheese manufacture and in being able to adjust these characteristics to the needs of the dairy industry. I agree with these goals. It has been gratifying to see the recent interest in the application of organisms that already have useable genetic "defects" such as those that are deficient in the production of enzymes to breakdown proteins and/or lactose. The proteinase negative (Prt-) mutants have been shown primarily to improve cheese yields while the lactase negative (Lac-) mutants have been helpful in cheese flavor improvement. I will discuss the uses of Prt- cultures which have been recently patented3.

Dairy lactic cultures are primarily valuable for the role they play in converting lactose to lactic acid at controllable rates. The organisms use lactose as a source of energy. Equally as important to them is a source of nitrogenous matter for them to produce cellular protein. Milk is a poor source of readily available protein building blocks. Only enough soluble nitrogen materials are present in fresh milk to allow lactic organisms to grow slightly more than one generation. Since we must have eight to eighteen generations during a bulk culture production cycle it is necessary to have proteolytic strains to dissolve casein. We have discarded Prt- mutants when they have become dominant in a culture and we have thus learned to rely upon their proteinase positive (Prt+) parent cultures. A cell of the latter has sufficient proteolytic enzymes to dissolve casein and provide soluble nitrogen for it's own growth and that of one to nine other Prt- cells4. It is obvious that dissolved casein will not produce as much of a dairy product as will intact casein. We will show that product losses associated with Prt+ cells can be as high as 10%!

Please interpret these percentages as intended. A 10% increase in cottage cheese does not mean going from a yield of 15 to 25 lb/cwt. We are not yet reporting how to make cheese out of water. We mean that the yield will go from 15 to 16.5 lb/cwt. Not as

Proteolysis vs. Yields. 3

dramatic but of significant economic importance to anyone making cottage cheese.

The highest yields obtainable in cottage cheese are from the intact casein that exists when direct acidification is involved\(^5,6\). Lower yields will be obtained wherever the proteolytic activities of lactic cultures are involved. The higher the proteolysis, the lower the yields. This explains why additions of higher nonfat milk solids do not always result in greater product yields. This also explains why lower yields per unit of casein result when nonfat milk solids are used in bulk culture production than when whey-based media are used\(^7\). It is logical that Prt- cultures ensure the most intact casein in curd products.

If high yields are obtained using Prt+ organisms it must be due to incorporation of protein fractions that do not play a primary role in coagulum formation, as with the incorporation of whey protein concentrates in Cheddar manufacture. Also, higher yields result when Prt+ cells are in such high concentrations that they are not under pressure to grow or proteolyze casein. Examples include pH controlled bulk culture or direct-to-vat bulk sets\(^1\).

The best model we have found to study yields has involved laboratory production of casein\(^6\). This is because the highest differences in yield are observed when the lactic cells are in contact with casein for long periods of time. We used 16 hours. There is less time involved in the production of short-set cottage and Cheddar cheese varieties so we would expect less of a measureable yield difference. Howard Heap, guest scientist from the New Zealand Dairy Research Institute, demonstrated that Prt+ cells of UC310+ produced yield losses in excess of seven percent over the casein yield with direct acidification (DA) (Figure 1).

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Figure 1 pictures the superior yielding properties of the less proteolytic organisms, designated by the minus signs, and it shows that the losses vary from one strain to another. Note that there were lower yields even with the Prt- strains than obtained with DA. The maximum difference between DA and strain UC310+ represents 1300 tons of casein imported annually in the U.S.A! The ability of the New Zealand dairy industry to capitalize on such observations explains why they lead the world in defined strain technology.

Gary Stoddard\(^8\) demonstrated that it was practical to add sufficient nitrogenous matter to bulk culture media to provide a carryover into cheese milk. This allowed most strains of Pri-cultures to produce acid at normal rates for the production of short-set cottage cheese. This study also demonstrated the greatest yield losses with strain UC310+. The yield increase associated with UC310-bulk culture amounted to 2.26%, enough to pay for the added nutrients in the bulk culture and provide a significant profit. Ekart

Proteolysis vs. Yields. 5

et al. 9 also found UC310+ to cause great losses in cottage cheese production (Figure 2).

In this figure the yield values from using UC310+ organism represent the zero line. Strain UC310- is not graphed because it agglutinated in the pasteurized milk supply and no yield data were obtainable. I shall talk more about this in a moment. Yields from strain UC77 were higher with the Prt+ strain producing 2% more dry solids and the strain UC77- producing an amazing 10.1% more dry solids than the UC310+. These are comparable to those differences reported earlier in Howard Heap's study and demonstrate even greater economic impact than our studies on cottage cheese. Note that the wet yield was 13.9% more than UC310+ or 2.8% more than the dry weight yield. This suggests either that the undissolved casein has higher water binding capacity than proteolyzed casein end products, that more whey protein is included in the curd, or both.

Proteolysis vs. Yields. 6

Sludge production in cottage cheese manufacture due to the agglutination of lactic cultures was found both by Ekart et al. 9 in their pilot plant studies at the University of Kentucky and by Fahad Khayat and Tod Scheuble 10 at Utah State University who used a new and sensitive test 11. We appreciated Randy Thunell warning us about this potential problem so we could screen our cultures. The Prt- mutants were found to be generally more susceptible to agglutination than the Prt+ strains. Khayat and Scheuble 10 examined 15 pairs of strains (Figure 3).

Figure 3

In this figure we have plotted the maximum dilutions of whey where agglutination occurred. Both S. cremoris and S. lactis are represented in these Utah isolates. Four strains caused agglutination even at 1/128 dilutions. Both isolates of UC310 agglutinated at


1/128 dilution. Remember that in the U. of Kentucky study only the Prt- strain agglutinated. We did not have UC77 comparisons. Khayat and Scheuble\textsuperscript{10} found only four isolates that were nonagglutinating, 21+, 45+, 45-, and 85+. Several other strains appeared to be low in agglutination reaction in this sensitive test and might be useable for cottage cheese manufacture. Obviously, other isolates need to be obtained and Jeff Kondo's work to control agglutination needs continuation and encouragement. We first intend to make cottage cheese in the pilot plant using strains 45+, 45-, 77+ and 77- to confirm the laboratory data and see if 45- can produce the yields reported for 77-\textsuperscript{9}.

Photomicrographs\textsuperscript{9} showed that the Prt- mutants tended to produce significantly longer cell chains than did the Prt+ strains which demonstrated mainly paired cells. Perhaps chain length morphology is linked and should be examined in addition to agglutination properties during the strain selection process. It is obvious that careful strain selection for cottage cheese manufacture should include more than yield producing capability.

Proteinase negative cells can be used in Cheddar manufacture without the same concerns about agglutination. Yield advantages, however, are not as well defined as with cottage cheese. This is probably due to the limited 30-60 minute time interval that the cells are in contact with the casein before the coagulum is formed and the fact they are not under as great a pressure to grow during Cheddar manufacture. Even so, O'Leary and Hicks\textsuperscript{12} measured individual strain differences that showed over 2% yield advantages for the use of Prt- mutants even when several types of bulk culture media were compared. Differences in recovery of cheese solids exceeded 3.1% between the lowest (UC77+) and the highest yielding strain (UC320-) (Figure 4).

Proteolysis vs. Yields.

If the yield value from UC320-, the highest yield producing culture, was set at 100% then the other strains produced cheese solids recoveries as indicated. These were not due to phosphates or other media non-cheese additives but were due to the type of single strain culture used in bulk culture preparation. As with the cottage cheese study the UC77 pair showed the highest yield differences, in this case 2.36%. But with Cheddar the UC77 pair produced the lowest yields! The higher yielding strains were probably not useable for cottage cheese because of agglutination problems (Figure 3).

We were not able to find significant yield advantages when UC73- was used to make Cheddar cheese in the pilot plant. It was also of concern that this strain produced a bitter flavor when the cheese was ripened but Yiadom-Farkye and Ernstrom have

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Proteolysis vs. Yields. 9
recently presented evidence that the off flavor was probably due to the way the chymosin was used. Winkel\textsuperscript{15} recently demonstrated that careful selection of Prt- cultures for Cheddar manufacture could produce yield increases of 0.37\%. The range of yield increases would be 14.8 lb per 40,000 lb vat with his work to over 90 lb with the earlier study\textsuperscript{12}. Winkel calculated a $63 per day yield advantage for a 300,000 lb/day plant. This would amount to more than $400 per day for the higher yielding strains\textsuperscript{12}.

Khayat and Richardson\textsuperscript{16} compared cell mass production and two chemical assays for quantitating the proteinase activity of lactic cultures. All methods effectively separated Prt+ and Prt- cultures. Typical results for the preferred trinitrobenzene sulfonic (TNBS) acid method are portrayed in Figure 5.

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure5}
\caption{Figure 5}
\end{figure}


Proteolysis vs. Yields. 10

The average Prt- organism utilized more color producing compound than it produced so the measurement is below the control sample at 0.0. The Prt+ cultures produced readings above the control. Examples of S. thermophilus and Lactobacillus bulgaricus are plotted for comparison. The high proteolysis of L. bulgaricus suggests potential concern over product yield losses. This may not be of concern in yogurt products where whey is not separated from the product or in Swiss cheese where limited inocula and short exposure times are involved. But it might pay to examine the impact of this organism upon the yield of Italian cheese varieties.

Adjustment of proteolysis in products like yogurt and buttermilk where whey is not separated might be used to change body characteristics of the products. Pope\textsuperscript{17} made sour cream and buttermilk and demonstrated curd strength differences as summarized in Figure 6.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{Strength differences in sour cream and buttermilk.}
\end{figure}

\textsuperscript{17} Pope, B.K. 1985. Curd formation by proteinase positive and negative lactic cultures in sour cream and buttermilk. Unpublished studies. Utah State University, Logan, UT.
Proteolysis vs. Yields

Increased cutting strengths were noted both in buttermilk (BM) and in sour cream (SC) when UC310- was used in place of UC310+. It was necessary to provide additional nitrogenous stimulant in the substrate to assure normal acid production rates of the Prt- strains and these additions did not adversely affect the flavor. The strength differences tended to be reversed where high heat treatment of the substrate was involved. For example, UC310+ produced higher cutting strength than UC310- if the cream was heated at 87°C for two minutes before cooling and inoculating. Thus with some conditions the additional proteinase might improve body characteristics in a manner similar to when chymosin is used at low levels in cottage cheese manufacture. We have not evaluated these modifications in yogurt manufacture.

Craig Oberg is working with Jeff Kondo in the evaluation of the plasmid profiles of these organisms. Different plasmids seem to be involved in different strains and their loss does not seem to always explain proteinase loss. A molecular rearrangement has been suspected. Craig is also working with Fahad Khayat to correlate the plasmid profiles with the end products of proteolysis. The organisms are incubated with casein and the end products are then chromatographed. The patterns that have been produced are different for the Prt+ and Prt- strains and show significant promise in helping to define the strains that may be ultimately selected for maximizing yield or flavor, for example. Brent Pope is working to evaluated the performance of Prt- cultures in ultrafiltration retentates. There appears to be no problem in getting them to produce acid at normal rates in these products. Any yield differences have yet to be studied.

I am grateful to all those cited in the references for the hard work involved in generating the data shared in this paper. I would also like to thank the Dairy Research Foundation, the Utah State Dairy Research Advisory Board, and the Utah Agricultural Experiment Station for funding this research over the past six years.

Well, there you have it. I have not been telling jokes! I hope that I have been successful in helping establish the potential impact of proper lactic culture selection upon curd yields. It is gratifying to see the interest shown by our culture suppliers in providing this technology to the dairy industry. It is difficult to demonstrate patience with those who feel that such yield observations are not important in the day of government surplus and market over-
Proteolysis vs. Yields. It behooves us to squeeze every ounce of product available out of the raw material for if we don't someone else will and thus deserve the benefit. My attitude is best reflected in a recent ITT poster displayed in the London Heathrow Airport walkway, "Without research, there is no future!"
INNOVATIVE TECHNOLOGIES
COMPUTERIZED PROCESS CONTROL FOR CHEDDAR CHEESE MANUFACTURE

P.M. Linklater and R.J. Hall

There is now an increasing awareness in large Australian cheddar cheese factories of the need for improved process control. The multiple batching system in the vats feeds a continuous cheddaring machine. This has forced the cheesemaker to end the vat stage at fixed time intervals so that curd is fed continuously to the cheddaring machine. Therefore the modern cheesemaker has lost an important control action - that is altering the time the curd is in the whey. This adds considerable complexity to process control in the large cheddar factories.

A survey of process control performance in four large factories served to define a number of process control problems. The make routines used in these factories showed wide variation in the typical pH at Pump at the end of the vat stage. The highest typical pH at Pump was 6.3 and the lowest typical pH was 5.9. In our survey we did not attempt to assess the quality of the cheese produced by these different routines, although a Pump pH of 5.9 is generally believed to be unsuitable for long hold cheese.

We also found variation in the process within a factory. The normal make routine in one factory used a Pump pH of 6.28, 175 minutes after Rennet. When a new starter was introduced the pH at Pump was 6.20, 160 minutes from Rennet. Part of the explanation for this is that Australian factories still use Titratable Acidity (T.A.) to monitor acid production. We found that there was a poor correlation between T.A. as measured by the operator, and our pH measurements. The unreliability of T.A. results makes it difficult to adjust quickly the process to obtain the required pH at Pump.

Another problem for managers in controlling manufacture is that our operators do not always record accurately details of manufacture on the hand written make sheet. For example in our survey we found one vat which our chart recorder showed was cooked at 48°C, but the make sheet showed was cooked at 38°C. We have even found operators who filled in the make sheet in advance of manufacture. Our factories need automated recording of critical data, preferably in the form of an electronic make sheet on a V.D.U.

STarter KINETICS

The laboratory work in this project included a systematic investigation of the kinetics of growth and acid production of a representative sample of single strain starters. I will discuss here only 3 results. It is generally believed that a higher cooking temperature would inhibit acid production in the vat. Experiments in laboratory scale cheese vats showed that the pH at the end of the vat stage was almost unaffected by cooking at 36, 38.5 or 40°C in the vat stage, but that acid production was reduced significantly in the curd cooked to 38.5 and 40°C, during the subsequent cheddaring stage. This result shows that manipulation of the cooking temperature has serious limitations in the process control of cheese making.

An investigation of the effect of temperature on the kinetics of growth and acid production of starter revealed an explanation for these results. Starter growth (as dry weight increase) and acid production were both measured for a culture grown in whey. The temperature profile was approximately the same as that used in most Australian factories.
The first effect of an increase in temperature was to increase slightly the rate of acid production, followed by a progressive decline. At the end of 2.5 hours these opposing effects led to about the same total acid production as would have occurred at 30°C, and the rate of acid production for all 3 starters was approximately the same at 36, 38.5 and 40°C. The growth rate of the temperature insensitive starter was reduced slightly, but the growth rate of the 2 temperature sensitive starters was reduced significantly at the higher temperatures.

In summary, the growth rate of normal starter is reduced by an increase in the cooking temperature, but the total acid production in the vat is largely unaffected. The rate of acid production is reduced in the subsequent cheddaring stage. This explains the observation that a variation in the cooking temperature had no significant effect on acid production in the vat.

Another unexpected feature is that the cheddaring temperature does not affect significantly acid production during cheddaring. The explanation is that starters do not grow during cheddaring; they only produce "uncoupled acid", the product of maintenance, but not growth. This was established by adding a radioactive tracer to the curd and measuring the uptake which would occur during growth. However there was no measurable uptake, and so growth was not detected.

These results provided the basis for our approach to process control in cheddar cheese manufacture. I have already argued that the cheesemaker has lost effective use of the time in the vat as a process control variable. A further problem is that variation in the cooking temperature has little effect on acid production during the vat stage. This means that variation in the inoculation rate is the most effective control variable available to the cheesemaker.

The use of the inoculation rate as a control variable involves "Feed forward" control. This requires a laboratory test which will predict the appropriate inoculation rate. We have developed a simple laboratory test which simulates the vat. "Today's starter" is added to two samples of "Today's cheese milk", in one at a rate slightly below and in the other at a rate slightly above the expected optimum inoculation rate. After the addition of the normal amount of rennet, the samples are poured into 6 test tubes which are incubated for 3 hours in a water bath fitted with a programmable temperature controller which produces the same temperature profile as the vat. The pH is measured by destructive sampling of these test tubes at half hour intervals. We have found that this test accurately simulates the pH development in the vat. The inoculation rate to give the required pH can be estimated by interpolation between the pH values for the larger and the smaller inoculation rates at the appropriate time after renneting.

A further key requirement is that every step in manufacture should be accomplished without deviation from the set routine. The milk, and more especially the starter volumes must be reliably metered. The temperature must be controlled accurately. The time for inoculation, renneting, cutting, cooking and pumping must also be held constant for each vat. The aim is to keep all the process variables constant, except for the inoculum size which is varied relative to starter activity. This runs the process "In the groove" and keeps it there.

COMPUTER SYSTEM

We have developed a computer system designed to monitor and display the cheesemaking variables. The total system consists of:

1. Sampling tube.
This is designed to deliver whey, free from curd particles, to the pH meter. The body of the tube provides quiescent conditions so that the denser curd settles out of the whey flowing up the sampling tube.

2. Manifold.
   This comprises a bank of 2 valves per vat which are automated to sample whey for pH measurement. The waste valve runs the whey to the drain and the sample valve admits the whey to the pump.

3. pH measurement.
   Whey is drawn from the manifold through a peristaltic pump to the pH electrodes.

   This acts as a "Front end" for the IBM. It collects data and provides control functions. It is programmed in MicroMacBasic and is virtually a computer.

5. IBM PC XT.
   Data collected by the MicroMac is sent to the IBM for storage and display. The IBM is the interface between the computer system and the operator. It allows the operator to find out what is happening in the vat and in the cheddaring stage.

How it works.
   As the vat begins to fill, the starter is pumped in. This step is sensed by the MicroMac and the time logged. When the milk covers the heating section in the vat the temperature controller brings the milk temperature up to the required setting temperature. The MicroMac senses the "Rennet" step and records the time. The IBM then displays the status of this vat as "Rennet". After setting the curd is cut and when there is sufficient free whey the sampling routine begins, controlled by the MicroMac.

Sampling routine.
1. A short blast of compressed air is admitted to the settling tube to remove coagulum.
2. The waste valve is opened to allow the whey level to rise slowly in the settling tube, leaving the curd behind.
3. The vat now enters the queue in the programme for pH measurement. When the vat reaches the head of the queue the MicroMac opens the sample valve and whey runs along the manifold to the peristaltic pump and then at a controlled rate to the pH electrodes.

   The whole system depends on obtaining accurate pH measurements. We have had some difficulty with drift in the electrodes but we are gradually reducing this problem. We have been able to make accurate pH measurement feasible by using 2 pH meters. Thus any deviation between the meters immediately alerts the operator that a problem exists and the meters are checked. The system also pumps buffer through the manifold every hour to check for pH drift.

Display of the results on the IBM.
   The operator obtains any information he requires from a series of displays by pressing a key on the IBM keyboard. The most frequently used information is on the mimic display which summarises the current situation in each vat. It shows, in different colors, whether the vat has reached the stage of inoculated, rennetted, sampled or finished. It also shows the current pH, temperature and time from rennetting.

   Individual vat displays are provided to give more detailed information on conditions in the vat from inoculation to Pump. This
includes:
1. Date and vat number
2. Time from inoculation to Rennet.
3. Rennet, clock time.
4. Pump on, clock time.
5. Relative time from renneting (taken as zero time) to present time.
6. Target pH.
7. Current pH
8. Target temperature.
10. Dry pH.

Control problems
A wide variety of process control problems have been identified using this computer system, for example high pH values at Pump caused by not enough starter being added to the vat. In many cases the curd was held too long in the whey. At the opposite extreme there were some vats to which too much starter was added giving a pH at Pump as low as 5.7.

These results show gross inaccuracy in the volume of starter added to the vat. However they also serve to illustrate the potential in more normal situations for varying the inoculum size up or down on subsequent vats, depending on whether the achieved pH at Pump is above or below the target pH line.

In another vat there was a long interval between inoculation and renneting and the starter grew vigorously. The pH at Pump was 5.75. These 3 examples illustrate poor process control. They are extreme cases, but we have found enough of them to cause concern.

The system also measures the pH of curd at mill. The sample must be prepared manually, although the MicroMac records the result automatically. Another measurement which has proved useful is the duration of the stoppage times on the cheddaring belt. A stoppage time of 30 minutes or longer is more frequent than expected. This is an important method of detecting mechanical breakdowns. The significance of the stoppage is that the curd is held too long in the vat or in the cheddaring stage. The resultant cheese generally would not meet quality standards at maturity.

Analysis of results
Our colleague George Browning has written a number of programmes to summarize and analyse the large volume of data collected by the computer for a period of a month, or whatever period is required. These include the calculation of the frequency distribution of the pH at Pump, time from Rennet to Pump, and cook temperature. Another programme provides a listing of all the prolonged belt stoppages. The management do not want information on vats which are manufactured within the process specifications. An exception report lists all vats which, for example, were below the specified pH at Pump. The programme gives the vat number, date and the variable outside the specification.

This simple system of summaries and exception reporting to management shows that the process is, or is not, running "In the groove". This reporting allows more efficient management of production. The first factory in which this system was installed consistently produced a proportion of poor quality cheese. The computer quickly showed the reasons.

1. Temperature control in the vat stage was inaccurate. This problem was solved by using the temperature control subprogramme in the
2. Addition of starter to the vat was inaccurate. Occasionally starter was missed completely. Furthermore starter from different bulk starter units showed different activities, probably caused by different setting temperatures.

3. Mechanical breakdowns held up production and delayed the cheese in the vat and in the cheddaring stage. The resultant cheese was often quite unsuitable for maturation. More effective preventive maintenance will improve this situation.

4. The cheese was often held too long in the vat. There was a mistaken belief that when acid production was slow the situation could be corrected by simply holding the vat until the correct pH was achieved. They no longer use these excessive times in the whey.

5. Some vats were filled only partially with milk and therefore the inoculation rate was effectively increased. This caused the pH at Pump to be too low. Eventually we found that this was caused by slow arrival of tankers which meant milk was not available to complete filling the vat.

The computer reports allowed management to identify numerous previously unrecognized problems and then to solve them. This has resulted in savings in this factory of $300,000 per year.

We are now planning the next installation in another factory. This factory has a problem with wide variation in pH and high levels of moisture in the final cheese.

SUMMARY

We believe the critical parameter in controlling the process is the inoculum size. The key step in monitoring the process is continuous pH measurement in the vat. It is equally important to monitor the time and temperature at which each step in the process occurs and to keep the process "in the groove". This can be done with the purpose designed computer system described in this paper. This system also provides detailed exception reports and summaries which show trends and perspective. These reports have given to management a powerful tool to improve process control in the factory.
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<th>TIME</th>
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<td>1</td>
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<td>1.00</td>
<td>32.0</td>
<td>150</td>
<td>38.0</td>
<td>6.20</td>
<td>6.10</td>
<td>3.30</td>
<td>30</td>
<td>5.20</td>
<td>5.3</td>
<td>-</td>
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<td>6.40</td>
<td>4.00</td>
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<td>32</td>
<td>6.20</td>
<td></td>
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<td>150</td>
<td>38.0</td>
<td>6.21</td>
<td>6.10</td>
<td>5.00</td>
<td>30</td>
<td>6.50</td>
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<td>SAMPLE</td>
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<td>6</td>
<td>SAMPLE</td>
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<td>8</td>
<td>SAMPLE</td>
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<td>32.3</td>
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<td>6.39</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>RENNENET</td>
<td>5.00</td>
<td>32.1</td>
<td>55</td>
<td>36.5</td>
<td>6.45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>INOC.</td>
<td>5.30</td>
<td>32.0</td>
<td></td>
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</tr>
</tbody>
</table>
LINEAR PROGRAMMING IN THE ALLOCATION OF MILK RESOURCES FOR CHEESE MAKING

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I. PROBLEM DESCRIPTION

Allocation of milk resources for cheese manufacturing has a significant impact on the economic well-being of a cheesemaker. Milk resource allocation decisions should be influenced by the market condition for both raw materials and finished products as well as the impact this decision has on cheese yield and quality. It is a complicated decision with many ripple effects with which a cheese manufacturer is faced with on a daily basis.

The milk resource allocation problem (MRAP) can be illustrated as follows: raw milk resources are aggregated in storage silos at the plant, additional milk resources are available in the marketplace for cheese manufacture. These resources include nonfat dry milk (NDM), condensed skim milk, skim milk, cream, or whatever else may be available. The cheesemaker's task is to formulate cheesemilk to make cheese that conforms to pre-established fat-on-the-dry-basis (FDB) standards. This is commonly called standardization.

To achieve this the chemical composition, namely the casein and fat percentages of the milk are to be adjusted to a ratio that will then produce a cheese with the desired FDB. This adjustment of casein and fat percentages may be made by adding fat or casein from another milk resource or by removing fat and casein in the form of cream from a milk resource or a combination of these.
The ability to combine options makes the selection of a milk standardization policy a complicated task. There are many factors about each option that need to be taken into consideration to optimally allocate the milk resources. This problem is a unique form of the blending problem encountered in many food formulations (1,3,4,7,8). The blending is considered when milk resources are added to the milk to adjust casein and fat percentages. The unique part of the problem is encountered when the option of cream removal is considered. Blending is typically and strictly a combination problem while the ability to remove cream makes it a multi-product decision.

The milk resource allocation problem investigated here is a focused problem facing cheesemakers. This problem may be defined specifically as:

Given a supply of milk resources, a fixed set of manufacturing conditions, a fixed market for raw materials and products, and the type of cheese to produce, what is the best way to assemble the cheesemilk to the required casein to fat ratio to produce the cheese desired?

Given this problem definition linear programming can be employed for its solution.

II. LINEAR PROGRAMMING
Linear programming is not computer programming. Linear programming is a operations research method developed in 1947 by George B. Dantzig, then part of a research group of the U.S. Air Force know as project SCOOP (Scientific Computation of Optimum Programs) (2). The extraordinary efficiency of this method together with high speed computers have made linear programming the most powerful optimization method ever designed and the most widely applied in the business environment. Present applications include: Least cost ice cream formulas, least cost feed formulas, least cost fuel formulations, etc.

A detailed discussion of linear programming will not be given here as there are abundant texts on the subject. (1,2,3,4,5,7,8). Selection decisions are to maximize an objective function:

$$Z = c \cdot x$$

subject to a set of constraints:

$$A \cdot x \leq b$$

where:

$Z =$ value of objective function. In this example net returns from a vat of cheesemilk.

$x =$ vector of decision variables whose component levels in the optimal resource allocation are to be solved. In this application they are the pounds of each resource to use or the pounds of cream to remove in the optimal formulation

$c =$ vector of net returns associated with each unit of each activity.

$b =$ vector of constraint values which are limits on the amount of resources into and out of the system, and also define the ratio of casein and fat in the cheesemilk formulation.

$A =$ matrix of technical coefficients that relate resource use to resource constraints.
The optimal solution is obtained by one of several iterative algorithms.

III  MATHEMATICAL MODELING

To use linear programming, the cheesemaking process can be modeled as a resource conversion process. The input resources are milk, skimmilk, condensed skimmilk, non-fat-dry-milk (NDM), and cream. These milk resources are converted into output products of cheese, separated whey and whey cream.

The relationship between input milk resources and output products can be analyzed using a mass balance assumption. The cheese, whey, and whey cream yield rates per 100 pounds of cheesemilk can be determined by use of a modified cheese yield formula (9). This formula is:

\[
\frac{[\text{FR}(F) + \text{CR}(C)] \text{SR}}{1 - W}
\]

where
- \( \text{CY} \) = cheese yield per 100 lb.
- \( \text{FR} \) = fat recovery percentage divided by 100
- \( F \) = fat percentage of standardized milk
- \( \text{CR} \) = casein recovery percentage divided by 100
- \( C \) = casein percentage of standardized milk
- \( \text{SR} \) = salt solids retention factor
- \( W \) = moisture percentage divided by 100

The whey cream yield and separated whey yield can then be determined by the following formulas derived from the cheese yield formula.

WHEY CREAM YIELD:

\[
\frac{(F - \text{FR}(F)) \ast \text{WFR}}{\text{WF}}
\]

where
- \( \text{WCY} \) = whey cream yield per 100 lb.
- \( \text{WFR} \) = whey fat recovery percentage divided by 100
- \( \text{WF} \) = fat percentage of whey cream divided by 100
SEPARATED WHEY YIELD:
SWY = 100 - CY - WCY

Now that the mathematical relationship between the input milk resources and the output products is established with these yield formulas, linear programming can be used to optimize the cheesemaking process.

DECISION VARIABLES:

The variables used in the linear programming model will represent the pounds of each milk resource chosen to formulate a vat of cheesemilk. Additional variables will be used to represent the pounds of cream to be removed from each milk resource that has the potential to have cream removed.

CONSTRAINTS:

1) The amounts of each resource used minus the amounts of cream removed must sum to the proper vat size.

2) The casein to fat ratio of the standardized milk must be in the limits required to manufacture a cheese of the desired FDB. This will require two constraints. The first constraint restrains the casein to fat ratio of the final cheesemilk formulation to be below the upper limit allowed. The second constraint restrains the casein to fat ratio of the final cheesemilk formulation to be above the lower limit allowed. When a specific FDB is desired, one equality constraint can be used that ensures the final cheesemilk is exactly the casein to fat ratio necessary.
3) The amount of cream to be removed from any milk resource cannot be more than is available in that resource. This will require one constraint for each resource that has the potential to have cream removed from it.

4) Additional constraints may be added to limit the amount that a milk resource may be used. This may be required due to limitations on the amount of a milk resource available or to require that a milk resource is not used beyond a pre-established limit to maintain the final cheese quality. Constraints may also be added to limit the upper or lower fat and casein percentage of the standardized cheesemilk.

OBJECTIVE FUNCTION:

Maximization of net returns per vat of cheese is a probable objective because it sets an upper standard for the manufacturer to strive for. The definition of net returns for this application is the difference between output product revenues and input milk resource costs.

IV. AN EXAMPLE

To illustrate how the cheesemaking process can be modeled a hypothetical example is presented. A cheesemaker has the following resources available with the compositions and costs indicated.
The type of cheese to be produced is Mozzarella with a 37% FOB. The cheese yield formula is 85% fat recovery, 96% casein recovery, 49% moisture, and a salt solids retention factor of 1.13. The cream separators are capable of removing 45% fat cream for both sweet cream and whey cream. Whey fat is recovered at a rate of 100%. The size of a vat is 10,000 pounds. The prices received for the output products are:

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>PRICE/LB.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHEESE</td>
<td>$1.31</td>
</tr>
<tr>
<td>CREAM FAT</td>
<td>$1.80</td>
</tr>
<tr>
<td>WHEY CREAM FAT</td>
<td>$1.60</td>
</tr>
<tr>
<td>SEPARATED WHEY</td>
<td>$0.00</td>
</tr>
</tbody>
</table>

Given this information about the market for raw materials and finished products, the manufacturing parameters, and the cheese yield formula, a linear programming model can be developed that accurately represents the cheesemaking process.

The decision to be made is how should the cheesemilk formulation be made to produce a cheese with a 37% FOB while maximizing net returns.

DECISION VARIABLES.
The decision variables will represent the pounds of each resource to use and the pounds of cream to remove from a milk resource that has the potential to have cream removed. The decision variables are:

<table>
<thead>
<tr>
<th>MILK RESOURCE</th>
<th>VARIABLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBS. MILK FROM THE SILO</td>
<td>X1</td>
</tr>
<tr>
<td>LBS. NFDM</td>
<td>X2</td>
</tr>
<tr>
<td>LBS. SKIMMILK</td>
<td>X3</td>
</tr>
<tr>
<td>LBS. CONDENSED SKIMMILK</td>
<td>X4</td>
</tr>
<tr>
<td>CREAM REMOVED FROM THE SILO</td>
<td>X5</td>
</tr>
</tbody>
</table>

In this example only the silo has the potential to have cream removed, and variable X5 is added to account for this option.

CONSTRAINTS

# 1) \[ X_1 + X_2 + X_3 + X_4 - X_5 = 10,000 \]

This constraint requires that the resources used minus the cream removed will sum to the proper vat size.

# 2) The constraint on the casein to fat ratio of the standardized milk can be determined by manipulations of the cheese yield formula. The desired FDB of the finished cheese is to be 37%. The cheese yield formula can be used to represent what the FDB of the cheese will be(6). Using the values in the cheese yield formula, FDB can be represented as:

\[
\text{FDB} = \frac{\text{FR}(F)}{[\text{FR}(F) + \text{CR}(C)]\text{SR}}
\]

where

- \( \text{FDB} \) = fat on the dry basis
- \( \text{FR} \) = fat recovery percentage divided by 100
- \( \text{F} \) = fat percentage of standardized milk
CR = casein recovery percentage divided by 100
C = casein percentage of standardized milk
SR = salt solids retention factor

This equation divides the total fat recovered in the cheese by the total solids recovered in the cheese.

The casein to fat ratio needed to obtain an FDB can be determined from this equation by knowing FDB, FR, CR, and SR, and then using any value for F and solve for C. If one(1) is used for F then the casein to fat ratio is determined directly.

In this example the equation will be:

\[
0.37 = \frac{0.85(F)}{1.13 + 0.96(C)}
\]

When F = 1, then C = 1.23.
Substituting 1 for F and solving the equation with respect to C, a casein to fat ratio of 1.23 is determined. This means that the standardized milk will need to have a casein to fat ratio of 1.23 to produce a cheese with an FDB of .37.

To write the constraint to represent casein to fat ratio, the casein percentage of the cream that is to be removed from the silo needs to be determined. The equations and procedure to determine the casein percentage of the cream removed are:

STEP 1)

Determine casein percentage of non-fat portion of milk by:
\[
C = \frac{CNF \times 100}{100 - F}
\]

where
- \( CNF \) = casein percentage in non-fat portion of milk.
- \( C \) = casein percentage of silo milk.
- \( F \) = fat percentage of silo milk.

Using the casein and fat percentages for the silo milk:

\[
\begin{align*}
2.43 & = \frac{2.52 \times 100}{100 - 3.5} \\
\end{align*}
\]

The non-fat portion of the silo milk contains 2.52% casein.

**STEP 2)**

Determine the casein percentage of the removed cream by

\[
CC = (1 - FC)CNF
\]

where
- \( CC \) = cream casein percentage
- \( FC \) = fat percentage of cream divided by 100

The fat percentage of cream removed is 45%, using this value and CNF of 2.52%:

\[
1.386 = (1 - .45)2.52
\]

The casein percentage of the 45% fat cream removed from the silo milk will be 1.386.

A constraint can now be determined to ensure the standardized milk will have the casein to fat ratio of 1.23. Only one equality constraint is needed since the final FDB required is to be 37%. The general format of the constraint will be:

\[
\frac{\text{casein percentage of standardized milk}}{\text{fat percentage of standardized milk}} = 1.23
\]
Using the casein and fat percentages of the resources available and the cream to be removed. The following equation can be written:

\[ 0.0243X_1 + 0.28X_2 + 0.0256X_3 + 0.056X_4 - 0.01386X_5 \\
1.23 = \frac{0.035X_1 + 0.01X_2 + 0.001X_3 + 0.0024X_4 - 0.45X_5}{0.035X_1 + 0.01X_2 + 0.001X_3 + 0.0024X_4 - 0.45X_5} \]

Carrying out the arithmetic the final equation will be:

\[-1.88X_1 + 26.77X_2 + 2.44X_3 + 5.3X_4 + 54.07X_5 = 0\]

This constraint will restrict the formulation to have a casein to fat ratio of 1.23, which should yield a cheese with a 37% FDB.

#3) A constraint is needed to ensure that no more cream is removed from the silo of milk than is available. The equation to determine the amount of cream available per pound of milk is:

\[ FC \times F = TC \]

where

- \( TC \) = total amount of cream per pound of milk.
- \( FC \) = fat percentage of cream removed.
- \( F \) = fat percentage of milk resource from which cream is to be removed.

The silo has 3.5% fat and 45% fat cream may be removed. The equation is:

\[ 0.078 = \frac{3.5}{45} \]

There is 0.078 lbs. of 45% fat cream available from each pound of 3.5% milk form the silo. The constraint to maintain the amount of cream removed is:

\[ X_1 \geq 0.078X_5 \]

rearranging the equation becomes:

\[ X_1 - 0.078X_5 \geq 0 \]
#4) Constraints may be needed to restrict the amount of milk resources used due to resource limitations or to maintain product quality. There will be no other restrictions needed for this example.

OBJECTIVE FUNCTION

The objective in this example will be to maximize net returns. The net return from one pound of a milk resource input into the cheesemaking process can be represented by the following general equation.

\[ NR = (CY \times CP) + (WCY \times WF \times WCP) + (SW \times SWP) - CL \]

where

- \( NR \) = net returns
- \( CY \) = cheese yield divided by 100
- \( CP \) = cheese price per lb.
- \( WCY \) = whey cream yield divided by 100
- \( WF \) = fat percentage of whey cream divided by 100
- \( WCP \) = price per pound of whey fat
- \( SW \) = separated whey yield divided by 100
- \( SWP \) = separated whey price per lb.
- \( CL \) = cost per lb. of input resource

The net return will be computed for each milk resource that is available for the formulation and a modification of the net return calculation will be used to estimate the net returns for each pound of cream removed from the silo. Only the net returns for the silo and the cream removed from the silo will be presented, the net return computations for the other three milk resources would be the same as for the silo except the respective casein and fat percentages, and cost would be used.
For the silo, the fat and casein percentages were 3.5 and 2.43 respectively. Using the yield formulas presented earlier, the cheese, whey cream, and separated whey yields would be:

CHEESE:

\[
\frac{[.85(3.5) + .96(2.43)]1.13}{1 - .49} = 11.76
\]

WHEY CREAM:

\[
\frac{(3.5 - .85(3.5)) \times 1}{.45} = 1.17
\]

SEPARATED WHEY:

\[
87.07 = 100 - 11.76 - 1.17
\]

Dividing the cheese, whey cream, and separated whey yields by 100, the yield rate and value for each pound of silo milk are:

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>YIELD</th>
<th>PRICE</th>
<th>VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>cheese</td>
<td>.1176</td>
<td>1.31</td>
<td>0.1540</td>
</tr>
<tr>
<td>whey cream</td>
<td>.0117 \times .45</td>
<td>1.60</td>
<td>0.0084</td>
</tr>
<tr>
<td>separated whey</td>
<td>.8707</td>
<td>0.00</td>
<td>0.0000</td>
</tr>
<tr>
<td>total</td>
<td>1.000</td>
<td></td>
<td>0.1624</td>
</tr>
</tbody>
</table>

0.1624 - 0.1199 = $0.0425 per lb.

The whey cream yield is multiplied by .45 to convert pounds of whey cream to pounds of whey fat, because cream is paid for on a pounds of fat basis. The return for each pound of silo milk will be $.1624. The cost was $.1199. The net return per pound of silo milk input would be $.0425. This procedure would be used to compute the value for each pound of the NFDM, skimmilk, and condensed
skimmilk. The net returns per pound of these milk resources are:

<table>
<thead>
<tr>
<th>RESOURCE</th>
<th>NET RETURN</th>
</tr>
</thead>
<tbody>
<tr>
<td>SILO</td>
<td>$0.0425</td>
</tr>
<tr>
<td>NFDM</td>
<td>$-0.1327</td>
</tr>
<tr>
<td>SKIMMILK</td>
<td>$0.0193</td>
</tr>
<tr>
<td>CONDENSED SKIMMILK</td>
<td>$0.0424</td>
</tr>
</tbody>
</table>

The net returns for the cream removed from the silo are computed as the cream revenues minus the value of the cheese, whey cream, and separated whey yields forgone. These yields are computed in the following manner. The fat percentage of the cream is 45 and the casein percentage was determined to be 1.386. The value of the foregone products from the cream can be determined from the yield equations presented earlier.

CHEESE

\[
87.7 = \frac{[0.85(45) + 0.96(1.386)]1.13}{1-0.49}
\]

WHEY CREAM

\[
15 = \frac{(45 - 0.85(45)) \times 1}{0.45}
\]

As is evident there is a positive mass balance with 87.7 + 15 = 102.7 lbs. of cheese and whey cream yielded from the 100 lbs. of cream. This is because the cheese yield formula is accurate only at reasonable fat and casein levels. This is inconsequential for the linear programming analysis since these products are not actually produced from the cream and only the fat in the whey cream is being
valued. The cheese and whey cream yield and value for each pound of cream removed is:

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>YIELD</th>
<th>PRICE</th>
<th>VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheese</td>
<td>.877</td>
<td>$1.31</td>
<td>$1.149</td>
</tr>
<tr>
<td>Whey cream</td>
<td>.15 x .45</td>
<td>$1.60</td>
<td>$0.108</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>$1.257</td>
</tr>
</tbody>
</table>

The whey cream is multiplied by .45 to convert the pounds of whey cream to pounds of whey fat. The $1.257 value for each pound of cream removed is to be considered as foregone earnings. In place of converting the casein and fat in the cream into cheese and whey cream, the cream is removed and sold as 45% fat sweet cream at a value of .45 x $1.80 = $.81 per pound. The net return contribution for each pound of cream being removed from the silo milk can then be estimated as $0.81 - 1.249 = $-.447.

An objective function representing the net returns from each pound of resource entered into the formulation and each pound of cream removed from the silo milk can be written as:

\[ 0.0425X_1 - 0.1327X_2 + 0.0193X_3 + 0.0424X_4 - 0.447X_5 \]

This is the equation that is to be maximized when values for the decision variables are determined. The constraints that characterize the cheesemaking system are again:

\[ X_1 + X_2 + X_3 + X_4 - X_5 = 10,000 \]
\[ -1.88 \times X_1 + 26.77 \times X_2 + 2.44 \times X_3 + 5.3 \times X_4 + 54.07 \times X_5 = 0 \]
\[ 0.078 \times X_1 - X_5 \geq 0 \]
\[ X_1, X_2, X_3, X_4, X_5 \geq 0 \]
A solution can be obtained by any one of a number of iterative algorithms available. When the model is solved the following formulation is obtained:

\[
\begin{align*}
X_1 &= \text{LBS. OF SILO MILK} = 7380 \\
X_2 &= \text{LBS. OF NFDM} = 0 \\
X_3 &= \text{LBS. OF SKIMMILK} = 0 \\
X_4 &= \text{LBS. OF CONDENSED SKIMMILK} = 2620 \\
X_5 &= \text{LBS. OF CREAM TO REMOVE} = 0
\end{align*}
\]

The objective function value will be $425. Using the cheese, whey cream, and separated whey yield formulas the product amounts and values for the 7380 pounds of silo milk and the 2620 pounds of condensed skimmilk are:

<table>
<thead>
<tr>
<th>AMOUNT</th>
<th>CHEESE</th>
<th>WHEY CREAM</th>
<th>SEPARATED WHEY</th>
</tr>
</thead>
<tbody>
<tr>
<td>SILO</td>
<td>7380</td>
<td>868</td>
<td>86</td>
</tr>
<tr>
<td>COND</td>
<td>2620</td>
<td>324</td>
<td>2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>10,000</td>
<td>1,192</td>
<td>88</td>
</tr>
</tbody>
</table>

The value of the cheese, whey cream, and separated whey is:

- Cheese: \(1,192 \times 1.31 = 1,561\)
- Whey cream: \(88 \times 1.60 = 64\)
- Separated whey: \(8,720 \times 0.00 = 0\)

Total value: $1,625

The cost of the resources used is:

- Silo milk: \(7380 \times 0.1199 = 885\)
- Condensed skimmilk: \(2620 \times 0.1201 = 315\)

Total cost: $1,200

The net returns are then determined as:

\[1,625 - 1,200 = 425\]
The standardized milk composition will contain 3.26% casein and 2.65% fat. This gives a casein to fat ratio of 1.23 which should yield a cheese with approximately a 37 FDB.

In this example labor and machine costs to perform the standardization were not taken into consideration. These types of costs vary widely from plant to plant. It is these considerations that the interaction between man and model must occur. The cheesemaker’s judgement is needed to evaluate the feasibility and economics of a solution proposed by the model.

V. SENSITIVITY ANALYSIS

Solving a linear program usually provides more information about an optimal solution than merely the value of the decision variables. For this example, information about the costs for the resources not included in the formulation would be valuable. The question to be answered is at what cost will a milk resource that is not in the optimal formulation replace a milk resource that is currently in the optimal formulation? This can be determined by evaluating the range for an objective function coefficient that is not in the optimal formulation. The computer solution will give a set of values called the "reduced costs". A reduced cost is the change in the value of the objective function per unit increase in the value of a decision variable(2). The reduced costs for this example are:
<table>
<thead>
<tr>
<th>RESOURCE</th>
<th>REDUCED COST</th>
<th>OLD COST</th>
<th>NEW COST</th>
</tr>
</thead>
<tbody>
<tr>
<td>SILO</td>
<td>0.0000</td>
<td>0.1199</td>
<td>0.1199</td>
</tr>
<tr>
<td>NDM</td>
<td>-0.1748</td>
<td>0.9400</td>
<td>0.7652</td>
</tr>
<tr>
<td>SKIM</td>
<td>-0.0231</td>
<td>0.0547</td>
<td>0.0316</td>
</tr>
<tr>
<td>CONDENSED</td>
<td>0.0000</td>
<td>0.1201</td>
<td>0.1201</td>
</tr>
<tr>
<td>REMOVE CREAM</td>
<td>-0.4034</td>
<td>1.8000</td>
<td>2.6964</td>
</tr>
</tbody>
</table>

Reduced costs can then be interpreted to give the change in cost or price, in the case of removed cream, of the resource before it will be cost effective enough to enter the optimal formulation. The silo milk and condensed skimmilk have a zero reduced cost because they are already in the optimal formulation. Non-fat-dry-milk has a -0.1748 reduced cost; this means that the cost for a pound of NDM would have to decrease from $0.94/lb to $0.7652/lb before it would replace a resource that is already in the optimal formulation. This is the same interpretation that skim milk will have.

The cream price per pound of cream would have to increase $0.4034/lb. before cream removal will be economically feasible. Presently the cream price is $1.80/lb. of fat. To convert the reduced cost for cream into what the price would need to be for a pound of fat the reduced cost is divided by the fat percentage of the cream. In this example 0.4034/0.45 = 0.896. The price for a pound of cream fat would need to be increased $0.896 to $2.6964/lb. before cream removed from the silo milk would be an economically feasible alternative.
VI. CONCLUSION

Linear programming can be an effective management tool in the allocation of milk resources for cheese making. This method can give insight and direction to the cheesemaker in: standardization methods, cheese composition to produce, milk resource procurement decisions, and milk pricing decisions.

VII. COMPUTER PROGRAM

A computer program entitled "Milk Resource Allocation Decision Support Program" has been written to perform this type of analysis. The program allows entry of up to eight resources, market prices for products, and manufacturing conditions. It will then formulate the mathematical model, solve the LP, and interpret the solution. This program is available through the Walter V. Price cheese research institute.

ACKNOWLEDGEMENTS

This research was supported by the Walter V. Price Cheese Research Institute at the University of Wisconsin, and the College of Agriculture and Life Sciences, University of Wisconsin, Madison.
REFERENCES.


Predicting Shelf-Life
Of Cottage Cheese By Impedance Microbiology

Dr. Charles H. White
Mississippi State University

The shelf-life of cottage cheese is shortened primarily by the same bacteria that shorten the shelf-life of our fluid milk products—psychrotrophic bacteria. Before we proceed, a couple of definitions would be in order:

Shelf-life--The actual number of days of refrigerated storage (at a specified temperature, e.g., 45°F) that the product remains good after packaging.

Psychrotroph--Bacteria capable of fairly rapid growth at 45°F (will grow better as temperature increases.)

Impedance--The total opposition to flow of an alternating current through a conducting material (for Bactometer purposes generally a microbiological growth medium.)

Impedance Microbiology--The study of electrical impedance variations resulting from the metabolic activity of growing microorganism.

Note: Most of what I will report to you if from a study done by one of my former graduate students and myself (Bishop and White, J. Food Protect., 1985, 48:1054-1057.)

Mohamed and Bassette (J. Dairy Sci., 1979, 62:222-226.) attributed cottage cheese vat failures to milk heavily contaminated with psychrotrophs regardless of whether the cheese was manufactured by the direct-acid-set method or by the conventional starter culture procedure. Average cottage cheese yields have been shown to decrease due to psychrotrophs. (Aylward, et al., J. Dairy Sci., 1980, 63:1819-1825; Hicks, et al., J. Food Protect., 1982, 45:331-334)
Therefore, psychrotrophic growth and subsequent metabolism are the focal points of shelf-life prediction rests. The test results must be available within a time period which would allow for effective corrective measures, preferably less than 48h. As a result of this need, the objective of this research was to investigate the use of bacterial numbers and their metabolites, after varying preliminary incubations, as indicators of the potential shelf-life of creamed cottage cheese. The word "potential" is a necessary term in any shelf-life study. Since the experiment progresses under ideal conditions, this would exclude temperature abuse, insanitary handling and other such factors that could possibly occur both in the home and the market place.

Cottage cheese was collected prior to creaming and artificially contaminated with psychrotrophs (Pseudomonas fluorescens.) All cheese was packaged and stored at 45°F. Bacterial enumerations conducted were: aerobic plate count (32°C for 48h; APC), psychrotrophic bacteria count 7°C for 10 d; PBC) (Std. Meth.) modified psychrotrophic bacteria count 21°C for 25 h; mPBC) (Oliveria and Parmalee, 1976, JMF Technol. 39:269-272) and gram-negative bacteria by crystal violet TTC agar (21°C for 72 h; CVT) (Compendium-Speck) Samples were subjected to preliminary incubation (PI) before bacterial enumerations, except for PBC. Preliminary incubations were none, 21°C for 7 h, 21°C for 14 h, 13°C for 18 h and 18°C for 18 h.

Impedance measurements were done with the Bactometer M120SC Microbial Monitoring System (Bactomatic, Princeton, NJ.) Modified plate count agar (MPCA) and modified CVT (MCVT) agar were used. For each cottage cheese container, one-half of the cottage cheese was aseptically removed and replaced with sterile plate count broth (Difco Laboratories, Detroit, MI.) The sample solution was then adequately mixed and PI was determined at
At the completion of PI, each sample was again mixed and 0.5 ml was inoculated into duplicate wells of each agar type. Impedance detection was done at 21°C. Average impedance detection time (IDT) values from each medium were compared to the shelf life of the sample tested.

Proteolysis of each sample was determined by the o-phthaldialdehyde method of Church et al. (J. Dairy Sci. 1983, 66:1219-1227). Endotoxin (lipopolysaccharide levels were evaluated by the gelation Limulus amebocyte lysate (LAL) assay (Difco Laboratories.) Cottage cheese (5 g) was blended with 5 ml of pyrogen-free Mg-saline (Tsui and Steindler, Appl. Environ. Microbial 1983, 45:1342-1350) and serial dilutions made thereof.

The shelf-life of each cottage cheese sample was determined by daily sensory evaluation. This value must be used as potential shelf life when making correlation comparisons to measured parameters. Some of the results of the study were as follows: Linear relationships to potential shelf life of cottage cheese at 7°C (Table 1) indicated that bacterial enumerations, even after preliminary incubation, were of little value for estimation of shelf life. All presented correlations were significant (P<0.05), but these values were too low with too much associated variation to be reliable initial indicator of cottage cheese quality. The gram-negative bacteria count on CVT agar had the highest correlation value (R) to potential shelf life, with -0.61 for the quadric effect. (Table 2).

Endotoxin (lipopolysaccharide) concentration was also significantly related to potential shelf life (P<0.01) as evidenced by the linear and quadric coefficients of -0.73 and -0.81, respectively. The gelation LAL method was used in favor of the spectrophotometric LAL method due to problems in clarifying the cottage cheese sample to obtain a relatively
particulate-free resuspension solution which is needed for the spectro-
photometric assay. By using the gelation method, values lacked specificity
due to their being dependent upon the dilution series. This may partially
explain the obtained correlations values being lower than previously ob-
tained with milk. Due to this effect, endotoxin concentration could not
be used to predict potential shelf life of cottage cheese, but could
possibly be used to "categorize" cottage cheese samples as to their po-
tential shelf life. Samples with ≤ 100mg/g indicated a potential shelf
life of ≥ 12 d, whereas ≥ 800 ng/g indicated a potential shelf life of
≤ 7 d. (Fig. 1)

Proteolysis was found to be significantly related to potential shelf
life of cottage cheese (P < 0.01). The linear correlation coefficient
was -0.86, with a slight increase in correlation for the quadric effect
of -0.87 (Table 2 and Fig. 2). Categorization was also possible utilizing
proteolysis, as < 220 µg/g indicated a potential shelf life of ≥ 11 d, and
> 260 µg/g indicated a potential shelf life of ≤ 9 d.

A prediction equation was formulated to establish a system for
categorizing potential shelf life of cottage cheese:

Shelf life = 71.17 - 3.90 (Proteolysis) + 0.05 (Proteolysis)², with
shelf life in days and proteolysis in µg glyvine-leucine/g, and a regression
coefficient of 0.75.

Impedance detection was also significantly related to potential shelf
life of cottage cheese (P < 0.01) as illustrated by linear correlation values
of 0.84 in MCVT agar and 0.86 in MPCA. There existed an increase in cor-
relation values for the quadric effect of 0.87 in MCVT agar to 0.90 in MPCA
(Table 2). The MPCA appeared to be superior to the MCVT agar. The quadric
relationship between IDT and potential shelf life of cottage cheese (Fig. 3)
could be used to "categorize" cottage cheese samples as to their potential
quality. One sample which presents somewhat of a problem, i.e., at 
(16 h, 6 d), was observed to have a defect due to an "unclean" taste 
thought to be associated with poor quality nonfat dry milk powder. With 
this sample included, an IDT 6 h indicated a potential shelf life of 9 d, 
and an IDT 16 h indicated a potential shelf life of 11 d. Without 
the sample in question, an IDT 13 h indicated a potential shelf life of dd d. 

Impedance detection was also useful as a predictor of potential shelf 
life of cottage cheese with an equation of:

Shelf life = -0.61 + 1.21 (IDT) - 0.018 (IDT)^2, with shelf life in days 
and IDT in hours, and a regression coefficient of 0.80.

Utilizing all parameters studied (Table 2), regression equations were 
formulated to estimate the potential shelf life of cottage cheese, with 
r^2=0.90 and 0.94, respectively. When only IDT and proteolysis were in-
cluded in the equation, the coefficient of determination was 0.90 whereas 
when a total of five components was included, the coefficient of determination 
was 0.94. From the two-component equation, it appears IDT and proteolysis 
are of value in predicting potential shelf life of cottage cheese, but the 
five component equation would tend to emphasize the value of impedance 
detection.

Conclusions of this study were as follows:
1. Bacterial enumerations, even after samples had been subjected to PI, were 
of little value in the estimation of potential shelf life of cottage cheese.
2. Endotoxin (lipopolysaccharide) level was related to potential shelf life 
of cottage cheese (R = -0.81), and could possibly be used to "categorize" 
cheeses as to their potential quality.
3. Proteolysis was an indicator of potential shelf life of cottage cheese 
(R = -0.87), and could also be used to "categorize" samples, as well as
estimate their potential quality.

4. Impedance detection was an indicator of potential shelf life of cottage cheese ($R = -0.90$), and could be used to "categorize" cheese sample.

5. Impedance detection in MPCA resulted in a relatively simple prediction equation for potential shelf life of cottage cheese: Shelf life = $-0.61 + 1.21 \text{(IDT)} - 0.018 \text{(IDT)}^2$. 
TABLE 1. Linear relationships ($r$) of bacterial counts to potential shelf life of cottage cheese at 7°C ($n = 65$ experimental units).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Linear enumeration</th>
<th>Bacterial enumeration</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td>PBC</td>
<td>APC</td>
<td>mPBC</td>
</tr>
<tr>
<td>None</td>
<td>-0.52</td>
<td>-0.53</td>
<td>-0.50</td>
</tr>
<tr>
<td>21°C, 7 h</td>
<td>-0.56</td>
<td>-0.50</td>
<td>-0.59</td>
</tr>
<tr>
<td>21°C, 14 h</td>
<td>-0.54</td>
<td>-0.53</td>
<td>-0.58</td>
</tr>
<tr>
<td>13°C, 18 h</td>
<td>-0.59</td>
<td>-0.57</td>
<td>-0.58</td>
</tr>
<tr>
<td>18°C, 18 h</td>
<td>-0.58</td>
<td>-0.51</td>
<td>-0.52</td>
</tr>
</tbody>
</table>

*PI, preliminary incubation.

bPBC, psychrotrophic bacteria count.
cAPC, aerobic plate count.
dmPBC, modified psychrotrophic bacteria count.
CVT, crystal violet TTC count.

TABLE 2. Linear ($r$) and quadratic ($R$) relationships of each parameter studied to potential shelf life of cottage cheese at 7°C ($n = 65$ experimental units).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Linear</th>
<th>Quadratic</th>
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</thead>
<tbody>
<tr>
<td>PBC</td>
<td>-0.52</td>
<td>-0.53</td>
</tr>
<tr>
<td>mPBC</td>
<td>-0.57</td>
<td>-0.59</td>
</tr>
<tr>
<td>APC</td>
<td>-0.59</td>
<td>-0.59</td>
</tr>
<tr>
<td>CVT</td>
<td>-0.59</td>
<td>-0.61</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>-0.73</td>
<td>-0.81</td>
</tr>
<tr>
<td>Proteolysis</td>
<td>-0.86</td>
<td>-0.87</td>
</tr>
<tr>
<td>mPTD-MCVT</td>
<td>0.84</td>
<td>0.87</td>
</tr>
<tr>
<td>mPTD-MPCA</td>
<td>0.86</td>
<td>0.90</td>
</tr>
</tbody>
</table>

*PBC, psychrotrophic bacteria count.

mPBC, modified psychrotrophic bacteria count.
APC, aerobic plate count.
CVT, crystal violet TTC count.
mPTD-MCVT, impedance detection time in modified plate count agar.
mPTD-MPCA, impedance detection time in modified CVT agar.

After corresponding preliminary incubation.
QUALITY TRENDS AND CONCERNS
SEVENTH BIENNIAL CHEESE INDUSTRY CONFERENCE

UTAH STATE UNIVERSITY

LOGAN, UTAH

AUGUST 26 - 28, 1986

THEME: QUALITY TRENDS AND CONCERNS

FDA CONCERNS ABOUT CHEESE AND CHEESE-MAKING PROCEDURES

JOHNNIE G. NICHOLS

REGULATORY FOOD CHEMISTRY BRANCH

FOOD AND DRUG ADMINISTRATION

WASHINGTON, D.C.
FDA CONCERNS ABOUT CHEESE AND CHEESE-MAKING PROCEDURES

The Food and Drug Administration (FDA) currently has a number of concerns about cheese and cheese-making procedures. These concerns have surfaced in part because of several recent foodborne disease outbreaks attributed to cheese and other dairy products.

Today I will be discussing some of our concerns such as:

1) The use of the 60-day aging period as an alternative to pasteurization of milk for cheese-making;
2) Adequacy of pasteurization;
3) Post-pasteurization contamination; and
4) I will be sharing with you some of this year's FDA dairy sampling results.

1) 60-Day Aging in Lieu of Pasteurization.

All natural cheese sold in the United States must either be made from pasteurized milk or be aged for 60 days or more at a temperature of not less than 35°F. In light of recent events, the FDA must reexamine this regulation. We are aware of the concerns of manufacturers of blue, aged cheddar, Swiss and other cheeses.
Although we are trying to be sensitive to their concerns, we will not be able to justify placing product attributes such as flavor over public health or safety considerations.

Where did the 60-day holding period requirement for cheeses made with raw milk come from? The requirement that cheese made from raw milk be held for 60 days, at 35°F or above, prior to sale was established as a result of the 1947 Administrative Hearing held to establish standards of identity for many natural cheeses, including cheddar cheese. The basis of the requirement seems to be twofold.

First, prior to the 1947 Hearing there was a body of research performed by several workers to determine how long pathogenic microorganisms exist in cheese. Apparently *Brucella abortus*, the causative agent of undulant fever or brucellosis, was the microorganism of concern because it is most often cited in the Hearing testimony. The research showed that *B. abortus* did not survive in cheese beyond 60 days.

Second, California passed a law in 1944 requiring that cheese either be made from pasteurized milk or be held at 35°F or above for 60 days before being sold. Other states and some municipalities followed suit. The law was a reaction on the part of the Public Health officials to several outbreaks of deadly disease which
occurred between 1939 and 1944 and were caused by ingestion of cheese. Mr. Edward H. Fredel, in testimony at the 1947 Hearing, attributed the outbreaks to the "stress of the war years," e.g., lack of cheesemakers, use of obsolete equipment. No disease outbreaks occurred after the end of the war when normal conditions of cheese manufacturing were resumed.

Salmonella, specifically Salmonella typhimurium, is mentioned only a few times in the testimony, but usually in reference to the fact that the microorganism exists in cheese beyond the 60-day holding period. Tucker, et al. (J.A.M.A. 131 (14), 1119, August 3, 1946) reported that S. typhimurium survived for 302 days in cheese, causing a 1945 outbreak of food poisoning in Kentucky, Tennessee and Illinois; a total of 384 cases were reported. The FDA reported that Salmonella from the same outbreak survived in small pieces of cheese for 198 days.

In general, it was the consensus of those who testified on this topic at the 1947 Hearing that the 60-day holding period at 35°F would adequately protect the public health. To better understand how this decision was reached, we must go back to the state of the art 40 years ago, rather than view it through the eyes of science in the 80s. Certainly, it was recognized at that time that certain pathogens such as Salmonella would survive this treatment; however,
it was believed that they would be weakened. Microbiologists of that era believed that it took a dose of thousands or perhaps even millions of Salmonella to cause illness. The 1985 Chicago outbreak proved that it actually takes less than one organism per gram of food to cause illness.

Public Health authorities in Ontario, Canada, seized cheddar cheese made from raw milk following a 1982 outbreak. This cheese caused 150 illnesses and one death. One lot contained viable Salmonella muenster after 125 days. Analysis of the raw milk revealed only one cow shedding Salmonella from three quarters of her udder. The cheddar cheese appeared normal, having a pH of 5.1, fat content of 35% and salt content of slightly more than 1%.

S. typhimurium was also found by Canadian authorities in cheese made from pasteurized milk contaminated with raw milk through a faulty flow-diversion valve. This outbreak resulted in 1500 confirmed cases of salmonellosis between January and July 1984. Salmonella survived in this commercial cheddar cheese for eight months.

Listeria received little attention prior to 1983, and it was not considered a major milkborne pathogen. The problem surfaced with an outbreak of listeriosis in Massachusetts in 1983 that resulted in 49
illnesses and 14 deaths. An epidemiological study implicated milk. No Listeria was found in pasteurized milk. However, Listeria monocytogenes was found in the raw milk supply from that area.

A major outbreak of listeriosis occurred in Los Angeles in the spring and summer of 1985, resulting in 142 illnesses and 47 deaths. The epidemiological studies implicated one manufacturer of Mexican-style cheese, and L. monocytogenes was confirmed in the product. Because of the Los Angeles outbreak, the FDA initiated a nationwide sampling program in the summer of 1985 and, as a result of this program, L. monocytogenes was identified in Liederkranz cheese manufactured in Ohio.

According to Dr. E. H. Marth of the University of Wisconsin, L. monocytogenes also has the ability to survive both the stirred curd and Camembert cheese-making processes. Researchers of the Food Research Institute, Madison, Wisconsin, inoculated pasteurized whole milk with $5 \times 10^2$ L. monocytogenes/ml; 3 strains (Scott A, V7 or California) were used to prepare a stirred curd-type cheese of normal composition which was ripened at 6 or 13°C. These 3 strains persisted at levels of >10 cells/gram for as long as 224, 196 and 126 days, respectively.
Camembert cheese was prepared from pasteurized whole milk inoculated to contain $5 \times 10^2$ *L. monocytogenes* (strains Scott A or V7)/ml. Results showed that *Listeria* counts for both strains increased to about $5 \times 10^3$ cells/gram of cheese within 24 hours after it was prepared. After proper mold development followed by 21 days of ripening at 6°C, there was no apparent change from the initial inoculation numbers of strain V7, whereas levels of strain Scott A decreased to 10 cells/gram. Rapid growth of strain V7 in cheese was detected after 35 days of ripening. Strain V7 reached levels of $1 \times 10^7$, $1 \times 10^7$ and $1 \times 10^5$ cells/gram, respectively, in wedge, surface and interior cheese samples taken after 56 days of storage. In addition, growth of strain V7 during this period paralleled the increase in the pH of the cheese to 7.0 during ripening.

2) Adequacy of Pasteurization

In April of 1986, Professor M.P. Doyle of the Food Research Institute, Madison, Wisconsin, reported the presence of viable *L. monocytogenes* in pasteurized milk after it had been processed through a commercial-scale HTST pasteurizer. Because of the continuing problems with *Listeria* in dairy products, the FDA is undertaking a research project to determine whether *Listeria* will
survive commercial pasteurization, either through the holding tube (inadequate thermal inactivation) or around the holding tube (airborne contamination).

The Twenty-first Session of the FAO/WHO Committee of Government Experts on the Code of Principles Concerning Milk and Milk Products met in Rome, Italy, during June 1986 to discuss the issue of proper pasteurization. The United States advised this committee that on the basis of the authority of the Federal Food, Drug, and Cosmetic Act, the Federal Import Milk Act and the Agricultural Marketing Act, the United States has promulgated regulations establishing the minimum time and temperature of heat treatment necessary to assure the destruction of pathogenic organisms and spores that are of public health significance in milk and milk products.

3) Post-pasteurization Contamination

Our investigations to date indicate that post-pasteurization contamination is the most likely cause of the contaminated dairy products that we have encountered so far this year. Most of these problems are solved by going back to basics, e.g., by checking floors, walls and ceilings rather than by looking for more exotic
solutions. For example, we have found problems caused by such things as cross-connections between raw and pasteurized milk or between sweet water and potable water, air supplies, time/temperature abuse of the product, use of contaminated raw materials, reworked product, etc.

Many of the pasteurizers under review by FDA field personnel are noted to have deficiencies. These include improper design, installation or operation. One of FDA's senior regional milk specialists recently reported that 15 of the last 16 pasteurizers that he reviewed at random failed to meet all the requirements of the Grade A Pasteurized Milk Ordinance. Failure of one of the built-in safety features on a pasteurizer for only a few seconds could let milkborne pathogens such as *Salmonella* or *Listeria* slip through and contaminate equipment, air handling systems, coolers, etc. Once established downstream from the pasteurizer, the microorganisms may serve as a focal point of finished product contamination for months.

The FDA is following up on the 1985 National Academy of Sciences/National Research Council (NAS/NRC) evaluation of the role of microbiological criteria for foods. This follow-up includes plans of action for hazard analysis and critical control point systems.
In establishing the minimum heat treatment necessary to assure the safety of the products from the viewpoint of public health, the most applicable heat-resistant pathogen and the composition of the product as well as the manner in which the product will be packaged and marketed (i.e., aerobic vs. anerobic status, and refrigerated vs. nonrefrigerated conditions) have been taken into consideration. Specific equipment and heat process requirements have been established to assure that every particle of product receives the necessary heat treatment to assure its safety.

The phosphatase test, turbidity tests, etc. are recognized as providing some indication, after the fact, that the products may not have been properly heat-treated or may have been contaminated with nonheat-treated product after adequate heat treatment was accomplished. However, these tests are not acceptable in lieu of properly designed and operated heat treatment equipment that performs the applicable minimum heat treatment required for the product.

Some of the United States' (FDA's) comments at the 1986 Rome meeting include the following:

a. It is unjustifiable that a premise of concern over product attributes would take any degree of precedence over the minimum heat treatment necessary to assure the safety of a product.
b. It is equally unjustifiable that a premise of minimum heat treatment is predicated on a philosophy of merely "minimizing possible health hazards arising from pathogenic microorganisms associated with milk and milk products." In essence, if the microbial load (pathogens) is below the level normally considered to be sufficient to cause illness or death in humans, the level is "satisfactory."

Considering the variables as to kinds of pathogens, human susceptibility to various pathogens, and the fact that milk and milk products are excellent media for microbial growth, coupled with the fact that there is no guarantee the products will be maintained at a temperature or condition that would prevent the multiplication of the pathogens, this philosophy is totally unacceptable from the viewpoint of public health.

c. Assurance of meeting minimum heat treatments cannot be predicated on "after the fact" tests of the finished product, rather than on requirements relative to proper heat treatment, equipment design and operation. Sole reliance on tests such as the phosphatase test is faulty since this test merely measures the average heat treatment received by all of the particles that comprise the product, rather than assuring that all of the particles have
received the minimum heat treatment necessary for complete
destruction of all pathogens of public health significance. False
positive results from the phosphatase test further create doubt
about the appropriateness of the test for its intended use.

4) Results to Date of the FDA's Dairy Initiatives

A summary of this activity is shown on the overhead chart.

As you can see from these results and the examples of foodborne
illnesses I've presented today, the FDA must carefully reexamine
each of these concerns. We must investigate many of the current
practices in light of new emerging microbiological problems, e.g.,
Listeria. In addition, we must analyze each of these concerns,
using our new knowledge of old problems such as Salmonella.
Effect of Milk Psychrotrophs on Flavor, Body and Chemical Profiles of Cheddar Cheese

Presented to: 7th Biennial Cheese Industry Conference
Utah State University

By: Dr. Charles H. White
Mississippi State University
Due to vast improvements in refrigeration and larger raw milk storage capacities, the age of our raw milk at time of cheese manufacture has increased rather dramatically over the past few decades. The major deterrent to extended shelf life in fluid milk and non-ripened cheese varieties is that group of bacteria referred to as psychrotrophs. These two factors, increased age of raw milk at time of use and psychrotrophic bacteria, go hand in hand in causing problems to the cheese manufacturer. When milk is maintained at less than 45°F, the primary type of bacteria that are able to grow are the psychrotrophs. The psychrotrophs for the most part are made up of members of the genus *Pseudomonas*, *Flavobacterium*, *Achromobacter* and *Alcaligenes*. All of these bacteria are gram negative rods which are capable of fairly rapid growth at refrigeration temperatures. It should be remembered that these bacteria grow optimally at ambient temperatures, grow steadily at refrigeration temperatures.

Much of the research which has been reported regarding the effect of psychrotrophs on cheese has been with regard to yield. Most researchers would agree that psychrotrophs do have a slight adverse affect on yield of Cheddar cheese. For this reason, many cheese manufacturers are adding a lactic starter culture to the raw holding tanks in order to retard the rapid growth of the psychrotrophs in the raw milk. A study which we conducted looked more at the effects of these psychrotrophs on the quality of the Cheddar cheese.
In addition, we attempted to define both chemically and using sensory methods exactly what was good and what was bad Cheddar cheese.

Manning (J. Dairy Res. 1979, 523-529) indicated that over 200 volatile compounds have been identified in Cheddar cheese but that little is known about the relative contribution they make to its flavor. He attempted to correlate gas chromatographic data with organoleptic evaluation. Marsili (J. Dairy Sci., 1985, 3155-3161) monitored concentrations of several key metabolites in Cheddar cheese during ripening in order to determine which compounds were the best predictors of the glycolytic, lipolytic and proteolytic age of the cheese. He found that the best predictors of the glycolytic age were propionic acid and acetic acid; the best predictors of lipolysis were the FFA C_{10}, C_{12}, C_{14} and C_{16}; and the best predictors of proteolysis were the free amino acids leucine, methionine and glutamic acid. He did not believe that headspace samplings with GC were good indicators of aging but that they did provide information about flavor.

Law (Adv. in the Micro. and Biochem. of Cheese and Ferm. Milk, 1984, Elsevier Appl. Sci.) illustrated the major flavor forming pathways in cheese manufacture as follows:
He indicated that high quality cheese is more likely to result from manufacturing conditions which allow the starter streptococci to use up virtually all the lactose. He reported on work by Turner and Thomas (N. Zealand J. Dairy Sci. and Technol., 1980, 265) which indicated that careful salting of the curd to a salt-in-moisture level as close as possible to 4.0% made this possible. He added that the role of the products of citrate metabolism was less clear. Diacetyl may be important. With regard to lipids, Law indicated that the role of lipolysis and FFA was more difficult to assess in cheeses whose ripening did not involve mold growth. Some researchers believe that the FFA are vital to cheese flavor while others indicate that the volatile fatty acids do not affect aroma of Cheddar cheese or very little at best. Law concluded by indicating that proteolysis (like lipolysis) proceeded based on the degree or stage of ripening.

With regard to non-sensory assessment of cheese quality, Manning et al. (In: Adv. in the Microbiol. and Biochem. of Cheese and Ferm.-Milk, 1984, Elsevier Appl. Sci.)

<table>
<thead>
<tr>
<th>Citrate</th>
<th>Lactose</th>
<th>Proteins</th>
<th>Lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fermentation</td>
<td>Lactic Acid</td>
<td>Peptides</td>
<td>Ketones</td>
</tr>
<tr>
<td></td>
<td>Acetic Acid</td>
<td>Amino Acids</td>
<td>Lactones</td>
</tr>
<tr>
<td></td>
<td>Diacetyl</td>
<td>Amines</td>
<td>Aldehydes</td>
</tr>
<tr>
<td></td>
<td>Acetaldehyde</td>
<td>S-Compounds</td>
<td>Fatty Acids</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>Thioesters</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Propionic Acid</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Law, Adv. in the Micro. and Biochem. of Cheese and Ferm.-Milk, 1984, Elsevier Appl. Sci.)
classified it into two types: (1) the assessment of quality by relating compositional analyses (% salt/moisture, % moisture/fat free solid, % fat/water free substance and pH) to cheese quality; and (2) determination of flavor compounds.

These workers also concluded that the Redox potential must decrease (a role of the starter bacteria according to Law et al., [1976, J. Dairy Res. 301] and Manning [1979, J. Dairy Res. 523]) in order to get the proper flavor compounds.

Using GCHS, these workers showed concentrations of volatiles typically found on mature Cheddar.

<table>
<thead>
<tr>
<th>Volatile</th>
<th>pg/ml</th>
<th>ng/ml</th>
<th>Volatile</th>
<th>ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen Sulfide</td>
<td>2,000</td>
<td></td>
<td>Acetone</td>
<td>10</td>
</tr>
<tr>
<td>Methanethiol</td>
<td>800</td>
<td></td>
<td>Ethanol</td>
<td>30</td>
</tr>
<tr>
<td>Dimethyl Sulfide</td>
<td>200</td>
<td></td>
<td>Butan-2-one</td>
<td>1</td>
</tr>
<tr>
<td>Pentan-2-one</td>
<td>1</td>
<td></td>
<td>Ethyl Acetate</td>
<td>0.5</td>
</tr>
</tbody>
</table>


With this background, we focused on the effect of psychrotrophs, i.e., bacteria that are capable of fairly rapid growth at refrigeration temperatures, in raw milk on flavor development of Cheddar cheese. Moreover, we tried to predict ripened cheese flavor by analysis of young cheese by establishing flavor profiles for Cheddar cheese rated "good" and "bad".
Specific methods were as follows:

1. Raw Milk and Cheese Preparation and Storage

   Raw milk was inoculated with one of two levels (10,000 and 100,000/ml) of one of two psychrotrophs (*P. fluorescens* 27; *P. fluorescens* 103) and incubated at 7°C for 48 h. The contaminated and control milks were pasteurized at 73.9°C for 17 sec. The Cheddar cheese was made in 378.4 liter vats following the normal cheese manufacturing procedure (11). The cheese was stored at 7°C and evaluated at 0, 5, 30, 60 and 180 d. Values obtained and analyses performed included: sensory evaluation (flavor and body/texture), gas chromatography with headspace samplings (GCHS) and high performance liquid chromatography (HPLC).

2. Sensory Evaluation

   Cheddar cheese from each vat was subjected to sensory evaluation by a four-member trained panel at 5, 30, 60 and 180 d. Modified ADSA Cheddar cheese scorecard was used to score the flavor (1-10 scale), while the body/texture was scored with 1-5 scale. A flavor score of less than 5 was regarded as unacceptable (poor), 5-6 fair, 7-8 good and 9-10 excellent.

3. Gas Chromatographic Headspace Sampling (GCHS)

   Samples for GCHS were prepared in duplicate as follows: A 2.0 g sample was weighed into a 10 ml vial and sealed by a hand crimper with special closures (Perkin-Elmer Corporation) comprised of butyl rubber
PTSE coated septum, star spring and aluminum cap. After sealing, samples were frozen and analyzed at a later date. Volatile organics such as acetone, 2-butanone, ethanol, 2-pentanone and propanol were monitored by gas chromatography with headspace sampling (GCHS).

4. **High Performance Liquid Chromatography (HPLC)**

Samples for organic acid analysis were prepared as described by Marsili. Organic acids (orotic, citric, pyruvic, lactic, formic, acetic and propionic) were measured by high performance liquid chromatography (HPLC).

Results are noted as follows:

1. **Effect of Psychrotrophic Contamination on Flavor, Body and Texture of Cheddar Cheese**

Mean flavor scores for the control and high psychrotrophic inoculation level (Table 1) revealed that the control cheese had significantly superior flavor scores than all the psychrotrophic treated cheese.

The predominant flavor criticisms in the cheese made from psychrotrophic contaminated raw milk were bitter and unclean after 60 d of refrigerated (7°C) storage (Table 2). By 180 d, bitterness was detected in 38% of the cheeses inoculated with both medium and high levels of psychrotrophs; none of the control samples were criticized as bitter (Table 3). This
indicates probable production of bitter peptides by the psychrotrophic bacteria. By 180 d, unclean flavor was detected in 25% of the cheeses inoculated with both medium and high levels of psychrotrophs. On the same day, none of the control samples were criticized as unclean (Table 3).

Mean body/texture scores for the control and high psychrotrophic inoculation level (Table 4) revealed that cheese made from psychrotrophic contaminated raw milk had significantly (P<.001) lower body/texture scores than the control. The predominant body/texture criticisms noted after 60 and 180 d were weak, open and gassy (Table 5).

2. Effect of Aging and Added Psychrotrophs to Raw Milk on Organic Acid Content and Flavor of Cheddar Cheese

Results of HPLC analysis are summarized in Table 6. Results reveal significantly (P<.001) higher levels of lactic and formic acids and lower levels of propionic acid in cheeses made from raw milk cultured with psychrotrophs than the control cheese. Although differences between the control and psychrotrophic contaminated cheeses were not statistically significant for the rest of organic acids, the control cheese had lower values than the psychrotrophic treated cheese for pyruvic acid content, and no trend was noted for acetic, citric and orotic acids.
3. **Effect of Aging and Added Psychrotrophs to Raw Milk on Volatile Organic Chemicals and Flavor of Cheddar Cheese**

Results revealed that control cheese had significantly (P<.001) higher ethanol value than the psychrotrophic contaminated cheese. Results indicated (Table 7) revealed a brief summary about the comparison of cheese made from psychrotroph-contaminated raw milk and control milk.

4. **Prediction of Flavor**

The dairy industry has an immediate need for a rapid method to predict the flavor of finished product.

Stepwise regression, parameters and their ratios on 0, 5 and 30 d were used to formulate regression equations to predict the flavor of Cheddar cheese at 180 d. Entering the moisture content at 0 d, streptococci counts at 5 d, pH at 5 d and acetic value at 0 d as shown (Table 8), a regression equation was formulated having a coefficient of determination ($R^2$) of .81. This means that 81% of the variation in flavor could be explained by these parameters. By increasing the variables, we were able to increase our $R^2$ to .997.

In order to use these equations to predict Cheddar cheese flavor after 180 d, variables of an individual Cheddar cheese sample would be substituted in the equation on given days. After computation, the resulting flavor score would be that predicted for the cheese after 180 d storage. Rather than actually using the
equations, one objective of this research was to identify the chemical components which best relate to the flavor of the Cheddar cheese. After establishing this point, then further investigation of these components should yield more definite conclusions as to their actual importance in flavor predictions.
Table 1. Effect of Psychrotrophic Contamination of Raw Milk on the Flavor of Cheddar Cheese

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Flavor Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Cheese</td>
<td>7.95 A*</td>
</tr>
<tr>
<td>P. fluorescens 103-10,000/ml</td>
<td>7.20 B</td>
</tr>
<tr>
<td>P. fluorescens 103-100,000/ml</td>
<td>6.90 B C</td>
</tr>
<tr>
<td>P. fluorescens 27-10,000/ml</td>
<td>6.65 C</td>
</tr>
<tr>
<td>P. fluorescens 27-100,000/ml</td>
<td>6.55 C</td>
</tr>
</tbody>
</table>

* Means not followed by the same letter differ significantly (P < .001).
Table 2. Most frequently noted flavor vs time for control and treated cheese.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5</th>
<th>30</th>
<th>60</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Flat</td>
<td>Flat</td>
<td>Flat</td>
<td>Acid</td>
</tr>
<tr>
<td>Psychrotroph</td>
<td>Flat</td>
<td>Flat</td>
<td>Flat</td>
<td>Bitter</td>
</tr>
<tr>
<td></td>
<td>Sl.</td>
<td>Bitter</td>
<td>Unclean</td>
<td>Acid</td>
</tr>
<tr>
<td></td>
<td>Bitter</td>
<td>Unclean</td>
<td>Sulfide</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Frequency distribution of flavor criticisms of control and psychrotroph treated cheese at 180 d.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. Samples</th>
<th>Flavor Frequency (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control</td>
<td>5</td>
<td>1(20) 0 0 4(80) 0</td>
</tr>
<tr>
<td>II. PF 103</td>
<td>10</td>
<td>2(10) 6(30) 5(25) 6(30) 1(5)</td>
</tr>
<tr>
<td>III. PF 27</td>
<td>10</td>
<td>3(14) 8(38) 5(24) 0 5(24)</td>
</tr>
</tbody>
</table>

*Percent values based on total number of defects and not total number of samples. More than one criticism could be used for each sample.
Table 4. Effect of psychrotrophic contamination of raw milk on the body of Cheddar cheese

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Body Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>4.20 A*</td>
</tr>
<tr>
<td>2. P. fluorescens 103-10,000/ml</td>
<td>3.65 B</td>
</tr>
<tr>
<td>3. P. fluorescens 103-100,000/ml</td>
<td>3.60 B</td>
</tr>
<tr>
<td>4. P. fluorescens 27-100,000/ml</td>
<td>3.35 C</td>
</tr>
<tr>
<td>5. P. fluorescens 27-10,000/ml</td>
<td>2.90 C</td>
</tr>
</tbody>
</table>

* Means not followed by the same letter differ significantly (P < .001).
TABLE 5. Most frequently noted body/texture criticism over time for control and psychrotrophic treated Cheddar cheese.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>1. Control</td>
<td>Open</td>
</tr>
<tr>
<td></td>
<td>Curdy</td>
</tr>
<tr>
<td>2. Psychrotroph</td>
<td>Weak</td>
</tr>
<tr>
<td></td>
<td>Open</td>
</tr>
<tr>
<td></td>
<td>Curdy</td>
</tr>
<tr>
<td></td>
<td>Crumbly</td>
</tr>
<tr>
<td></td>
<td>Gassy</td>
</tr>
</tbody>
</table>
Table 6. Relationship of organic acids in control vs. treated cheese.

<table>
<thead>
<tr>
<th>Acid</th>
<th>Control Lower Than Treated Cheese</th>
<th>Control Higher Than Treated Cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>Formic</td>
<td>Propionic</td>
</tr>
<tr>
<td>Citric</td>
<td>Lactic</td>
<td></td>
</tr>
<tr>
<td>Orotic</td>
<td>Pyruvic-NS</td>
<td></td>
</tr>
</tbody>
</table>
Table 7. Comparison of cheese made from psychrotroph-contaminated raw milk and control cheese.

<table>
<thead>
<tr>
<th>Higher than Control</th>
<th>Lower than Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate (NS)*</td>
<td>Acetate (P &lt; .001)</td>
</tr>
<tr>
<td>2-Butanone (NS)</td>
<td>2-Pentanone (NS)</td>
</tr>
<tr>
<td>Proteolytic (P &lt; .001)</td>
<td>Propionate (P &lt; .001)</td>
</tr>
<tr>
<td>Propanol (P &lt; .05)</td>
<td>pH (P &lt; .001)</td>
</tr>
<tr>
<td>Lactate (P &lt; .001)</td>
<td></td>
</tr>
<tr>
<td>Formate (P &lt; .001)</td>
<td></td>
</tr>
<tr>
<td>Moisture (P &lt; .001)</td>
<td></td>
</tr>
</tbody>
</table>

*Significance level in parentheses
Table 8. Regression coefficients for equation\(^2\) to predict ripened Cheddar cheese flavor\(^1\) by analysis of young cheese.

<table>
<thead>
<tr>
<th>Variable</th>
<th>b Value</th>
<th>Prob. F</th>
<th>R(^2)</th>
<th>Mean Flavor Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>58.604</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X1 Moisture (day 0)</td>
<td>-1.386</td>
<td>0.0001</td>
<td>0.81</td>
<td>6.5</td>
</tr>
<tr>
<td>X2 Streptococci (day 5)</td>
<td>-1.446</td>
<td>0.0016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X3 pH (day 5)</td>
<td>2.506</td>
<td>0.0009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X4 Acetic (day 0)</td>
<td>0.014</td>
<td>0.0590</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Flavor at 180 d.

\(^2\)Regression equation calculated as follows:

Flavor score = (58.604) + (-1.386)(X1) + (-1.446)(X2) + (2.506)(X3) + (0.014)(X4)
In conclusion, the aroma and flavor quality of Cheddar cheese was attributed to a delicate balance of organic acids and volatile organic chemicals produced as metabolites by culture bacteria during fermentation. The quality of ripened cheese at 180 d was predicted by analysis of young cheese.
"Improved Cheese Yields by Controlling Psychrotrophs in Raw Milk"

R. L. Sellars, D. McCoy and S. Lutzke

Chr. Hansen's Laboratory, Inc., Milwaukee, WI

7th Biennial Cheese Conference, Utah State University

August 26-28, 1986

INTRODUCTION:

In today's market much of our Grade A milk supply in North America may be "Too Clean" - microbiologically. This view has also been expressed by Dr. Kozak of FDA. We realize this statement is contradictory to what many professionals have devoted their professional careers. And, it seems ludicrous, under the circumstances, that such a statement would be made. You deserve some explanations as to why we feel this way. We hope you may have a better appreciation for our views, as to "why", at the conclusion of this presentation.

Many changes, which continuously exert economic and public health hazard pressures on manufacturers of dairy foods or processors of milk, have taken place in our industry in the past twenty-five years. These changes, resulting from new knowledge, new innovations and creative inventions or practices have influenced directly our economics in processing, manufacturing or producing dairy products of value. Without question these developments have reduced manufacturing costs. However, at the same time we may have created a serious potential health hazard that may not be easily resolved. Often, as in the laws of chemistry and physics, for every action there is a reaction.

Throughout our development and commercialization of the new developments, e.g., animal health and feeding practices; milk handling and storage on the farm; transport and storage of milk in upright silos; high
volume processing and the degree of heat treatment of milk and its delivery to cheesemaking vats; the mechanized-automated equipment and the cheese manufacturing process; the external pH controlled starter systems with defined, more phage resistance strains; the cheese curd stirred or cheddared mechanically and automatically packed into barrels or 640 pound blocks; aged and cured at different temperatures with or without compatible enzymes and/or specially treated cultures to develop and accelerate flavor, body and textural development; and, packaging, warehousing, sales and distribution — all have had a measurable and, in some instances, a tremendous effect upon the cheese industry. Most of these new practices have been positive. However, some of them have had a negative effect for some operations from time to time. One of these negatives has been the collection and storage methods of raw milk.

Those professionals, who were responsible for and directly involved in these developments, are to be commended and congratulated for their accomplishments. Much has been accomplished. However, in our collective perceptions that milk quality would be improved by effectively reducing the microbiological level (total plate count) to ever decreasing maximum levels, e.g., by lowering the maximum allowable standard plate counts of Grade A raw milk from 300,000 to 30,000 as we have done over the years, we did not understand clearly what our data was telling us. For example: why did our cheese yields continue to decrease during the early years as we processed more "bulk tank milk" than "canned milk" when no other changes were made? Why did this happen when our milk supply "appeared" to be better microbiologically, as shown by the reduction in our standard plate counts of incoming raw milk?

To be sure there were other changes over the years, e.g., use of different coagulants — more microbial than calf rennet or blends of calf and porcine pepsin — and in the cheese make schedules themselves, e.g., shorter...
make times. At the same time, subtle but definite changes were occurring in our raw milk supply. We saw the country shift from less high total solids milk coming from the Jersey, Guernsey and Ayrshire breeds to predominately the Holstein herds. We reasoned that the reduced casein and less butterfat was primarily the reason for the lower cheese yields. Yet, the yields remained high even when butterfat was removed for standardizing milk so our percentage of FDB's were not much over the legal limit of 50%. With the advent of larger, mechanized and automated vats monitoring of unseparated whey fat has become a standard practice to tell us how well we are doing and whether our yields will be as expected. However, at the same time, too often we failed to recognize some of the basic reasons why fat will separate from the coagulated casein. For example: if the amount of casein in the milk is insufficient to trap the fat in the casein matrix at coagulation, excess fat will be in the whey. Standardizing the cheese milk on a casein to fat ratio of 0.70, as proven by Price and Van Slyke several years ago, will effectively reduce unseparated whey fats. To be sure, the type and amount of coagulant used and how well the vat is cut will affect fat separation and cheese yields.

There are other factors than those mentioned which cause higher unseparated whey-fats and affect lower yields even when unseparated whey-fats may be reasonable, e.g., psychrotrophic contamination.

If the casein and fat have been altered or degradated by the activity of psychrotrophic proteinases and lipases, significant losses in cheese yield can occur; and, the quality of aged, mature cheese often is inferior when objectionable off-flavors such as bitterness and/or rancidity are present. Or, the overall flavor profile may not be acceptable. Either way, losses do occur. However, a primary economic loss is in cheese yield due to alteration of casein by psychrotrophic proteinase enzymatic activity during storage of the milk on the farm, during transport and/or storage at the plant.
PSYCHROTROPIC CONTAMINATION IN RAW MILK

Low Temperature Refrigeration

Low temperature refrigeration to prolong the "sweet" shelf life of raw milk on the farm and in the plant for two to four days or longer has dramatically changed the indigenous microflora associated with raw milk over the years. When raw milk was stored, collected and delivered daily to factories in cans, the predominant microflora was lactic-acid producing bacteria. Lactose is the primary milk component utilized by this group. In bulk tank milk the lactic type and total numbers have shifted more to the psychrotrophic group. Casein is the principle milk component degraded by heat resistant proteinase enzymes elaborated by many psychrotrophic bacteria during storage. These enzymes can cause the formation of dipeptides, which, if not further broken down into amino acids, can cause bitterness when accumulated to detectable levels. Degradation of casein to any degree directly affects cheese yields, e.g., more casein solids and fat losses in the whey.

Microbial Population in Raw Milk

A typical example of the microflora shift in raw milk over the years is illustrated in Table 1 (17,18). Low temperature refrigeration has directly and dramatically caused this undesirable shift. You will note the decrease in the population of mesophilic (lactic acid) types to the psychrotrophic group when raw milk is stored under low temperature refrigeration.

Table 2 (2) illustrates the population of four (4) individual raw milk samples initially and how they developed when stored at 40°F up to 96 hours. Samples 1 and 2 are reasonably low in psychrotrophic numbers initially. However, the bacteria in sample one reproduced significantly during storage while sample 2 did not. Sample 3, while reasonable initially, continued to
develop high numbers during storage. The psychrotrophic population in sample 4, while high initially, was exceptionally high at the end of four days. Without question, cheese yields and cheese quality would suffer if milk contaminated with these levels were used to manufacture cheese products. When psychrotrophic populations approach 100,000 CFU's/ml detectable levels of increased soluble non-protein nitrogen are observed. When this occurs there is evidence of enzymatic proteolysis and signifies a potential loss in yields.

The explosive growth by psychrotrophs in milk between 24 and 48 hours of storage as shown in Table 2 could be related to the concentration and the availability of nitrogenous compounds resulting from proteolysis. Nitrogen is necessary for cell synthesis.

**Specific Growth Rates**

An example of the differences in growth rates of psychrotrophs in Grade A milk and manufacturing grade milk were reported by Stofer and Hicks (19). This is illustrated in Figure I. They observed that while the Grade A milk sample had appreciably less psychrotrophic organisms initially, the specific growth rate (e.g., increase in cell numbers) was significantly greater in Grade A milk than in manufacturing grade milk. When stored two days, the population in Grade A milk increased by approximately three logs whereas the manufacturing milk increased only one log in two days of storage. During further storage, the psychrotrophic population continued to increase at a faster rate in the Grade A milk than in the manufacturing milk. Documentation as to why was not presented. However, the authors suggested that the higher numbers of lactic acid bacteria in the manufacturing grade milk was inhibiting psychrotrophic development during storage. Later, we will discuss the reasons we believe the lactics were responsible for retarding psychrotrophic development.
Moisture Retention

When casein in raw milk is hydrolyzed to any degree by psychrotrophic proteinase activity, changes in the molecular structure of casein and its hydrophobicity (water retention) within the coagulated casein matrix is appreciably changed as illustrated in Figure II (19). The psychrotrophic infected milks, Grade A and Manufacturing Grade illustrated in Figure I, were processed into cheddar cheese during the storage period. The only difference in these experiments was the age of the stored milk. The results clearly show the effects of storage and psychrotrophic contamination of these milks upon moisture retention in the cheese curd. The effect of heat treatment on partially hydrolyzed casein has yet to be fully explained. However, published reports have documented some of the changes in the hydrophobic nature (water retention ability) of degraded casein. Body and textural differences were particularly noted (9).

RECOGNIZABLE DEFECTS

pH

In most samples where appreciable casein degradation has occurred, one observes an elevation (higher) in pH as shown in Table 3 (2). During casein hydrolysis ammonia is released, thereby causing the milk to become more alkaline. Cheese manufacturers would be advised to monitor pH values of their raw milk before processing, not only to determine a possible significant psychrotrophic infection but also to alert them that an adjustment in coagulant usage may be advised, depending upon the pH and type of coagulant being used. Also, if one does not run starter pre-activity assays on the silo milk, a pH on the raw milk could give some indications of the starter usage rate for that particular milk and starter combination in order to maintain the same uniform make schedule.
Since many of the common non-pathogenic psychrotrophs also elaborate lipase enzymes (fat splitting) as well as proteinase enzymes (protein-casein splitting), one often observes higher ADV values (acid degree values) in milk with appreciable psychrotrophic infections as shown in Table 3 (2). Monitoring ADV values in raw milk should be done routinely to check for any abnormality of incoming raw milk. Higher values than normal often indicates an abnormal psychrotrophic infection and should alert operators of potential problems. By assaying raw milk for both pH and ADV's indications of potential problems are obtained long before psychrotrophic populations can be measured.

**Increased Coagulation Times**

When the pH and ADV values are higher than normal due to psychrotrophic infections, coagulation (or "set") times may be decreased or increased as shown in Tables 3 and 4 (2). As shown in these tables, when populations of psychrotrophic organisms increase appreciably, the ADV's and pH changes thereby influencing "set times". A change of 0.01 unit in pH of the milk is sufficient to cause a change in "coagulation and "set times", if compensations are not made. Lipolytic flora produce higher ADV values or more free fatty acids. Free fatty acids interfere with coagulant action causing slower sets. Proteolytic flora may partially digest the casein resulting in a fast, brittle set.

**HEAT STABILITY**

A number of publications have reported the stability of the proteinases and lipases that are elaborated by psychrotrophic bacteria both of which affect cheese yields and final product quality. These enzymes have demonstrated to be some of the most heat resistant enzymes known to man. Selected data is shown in Table 5 (5). Both enzymes withstand legal
pasteurization temperatures. Therefore, if they are present in appreciable quantity in raw milk, there will be residual activity in manufactured dairy products. Your attention is called particularly to the stability of the proteinase enzymes. When present in detectable quantities, cheese yields will be reduced. Similar data may be found in the literature. The data reported by Downey suggest strongly that even UHT processing would not inactivate these proteinases to any degree to prevent some losses in cheese yields.

**Yield Losses**

Losses in cheese yield due to the presence of active proteolytic psychrotrophs in raw milk have been documented in several technical publications. A brief summary of selected reports are presented in Tables 6 and 7 (1,12,13). Generally, slightly higher losses have been reported for cottage cheese manufacture than for American cheese varieties. This is believed due to the favorable fermentation times wherein the proteolytic enzymes continue proteolysis longer before coagulation.

Nelson and Marshall (13) reported that eight of nine strains of Enterobacter did not affect cheddar cheese yields (Table 7). However, when the population of one Enterobacter strain approached 200,000 CFU's/ml in milk stored at 5°C (41°F) a loss did occur. In 1977 Hicks, et al (7,8) reported cheddar cheese losses of up to 0.5% of maximum theoretical yield recoveries per day of raw milk stored at 5°C (41°F). Later (1983) in a separate study Stofer and Hicks (19) presented data showing yield losses of 0.6% per day of the maximum theoretical yield when psychrotrophic contaminated milk was stored for two to four days at 5°C (41°F).

As stated earlier, a potential loss in cheese yield can be predetermined by assaying raw milk for the non-protein nitrogen content. Extended proteolysis by psychrotrophs increases the amount of non-protein nitrogen in
the milk. For further verification of losses in yield during cheese manufacture, assaying for the non-protein nitrogen content in cheese whey will show the effect of psychrotrophic proteolysis. This is illustrated in Table 8 (2). Increased numbers of proteolytic psychrotrophs in raw milk will increase the non-protein content in cheese whey. Yield losses are directly proportional to the increase in non-protein nitrogen content.

**LACTO-PEROXIDASE SYSTEM (LPS)**

How and why has our raw milk supply frequently and more recently become contaminated with psychrotrophic bacteria — non-pathogens and pathogenic types? Are the groups of microorganism, particularly the pathogenic types new, e.g., *Listeria*, *Campylobacter*, *Yersinia* and *Enteropathogenic E. coli*? Why have they become more frequently detected in our raw and pasteurized milk products whereas in previous years there appeared to be no severe problems? Obviously, one reason for this is we are looking for them more carefully.

A natural bacterial inhibitor system is present in raw milk. And, it is activated by natural occurring lactic bacteria which produce micro-quantities of hydrogen peroxide during raw milk storage at temperatures sufficient to induce metabolic activity of the lactic organisms without any appreciable increase in lactic cell numbers.

This inhibitor system is called LPS (lacto-peroxidase system). This system is also prevalent in other biological fluids, e.g., saliva, tears, colostrum, etc. And, its mode of action is similar whenever it is activated.

The LPS is a complex system involving several reactions. Hesse (3) reported on this system in 1894. Much research of this natural, nonspecific, antibacterial system, which is present in bovine milk and other important biological fluids and natural foods, has been conducted over the years.
An active LPS consists of three components: a) The enzyme lactoperoxidase (LP); b) Thiocyanate (SCN\(^-\)); and c) Hydrogen peroxide (H\(_2\)O\(_2\)). The first two components are always present in milk in micro-quantities. The amount often varies with the stage of lactation. Hydrogen peroxide (H\(_2\)O\(_2\)) is missing and must be supplied by organisms which metabolize in milk, particularly at refrigeration temperatures. There is evidence that iodine can replace thiocyanate in this reaction (15).

A simplified reaction and action of LPS as described by Giesecke (6) is illustrated in Figure 3. The LP enzyme catalyses the oxidation of the thiocyanate ion (SCN\(^-\)) in the presence of hydrogen peroxide (H\(_2\)O\(_2\)). It has been suggested that the active antibacterial agent is the intermediary hypoiodite ion product. Some investigators have postulated that the short-lived oxidation product O\(_2\)SCN\(^-\) is somehow involved in the antibacterial effect (6). According to research results by (Bjorck, 1977, Reiter and Hamulv 1982) the LPS has bacteriocidal activity against Gram negative bacteria; e.g., Eschericia, Salmonella, and Pseudomonas, particularly, all of which have been isolated from refrigerated raw milk. However, in contrast to other broad spectrum activity of antibiotics, the LPS does not kill or inactivate lactic acid bacteria, or other useful microorganisms, which normally inhibit the gastrointestinal tract. In fact, LPS has been shown to actively promote lactic acid bacteria in milk and in our gastrointestinal systems. Also, another important fact is that there is no evidence to date that bacteria, e.g., the psychrotrophs or lactics, develop resistance to LPS since it is a natural self-regulating system that has been in existence for a long time.

The naturally occurring defense mechanisms for protection against pathogenic microorganisms, e.g., bacteria, yeast and viruses, such as the LPS
and Lactoferrin (LF), is receiving today much attention by researchers. For further understanding the chemistry and biological significance of LPS and other natural defense systems against pathogens you are referred to an excellent review book, "The Lactoperoxidase System", K. M. Pruitt and J. O. Tenovuo, Editors; Published by Marcel Dekker, Inc., New York 1985.

**RMI**

How can we then capitalize on the significance of the LPS in milk to control psychrotrophic infections in raw milk without any drastic or expensive change in current practices of handling milk? One way is to add appropriate and approved lactic acid bacteria to raw milk. The selected bacterial cultures must be able to elaborate micro-quantities of hydrogen peroxide in raw milk during storage at refrigeration temperatures, 40-45°F. This technique is called Raw Milk Inoculation.

**Process**

This process (Figure 4) as promoted by Chr. Hansen's Laboratory, Inc. (Milwaukee) involves the addition of super-concentrated, hydrogen-peroxide producing lactic acid culture cells to raw milk when it is received at the cheese plant. The lactic culture that is used for this purpose has been selected for: a) its hydrogen-peroxide producing ability in raw milk stored at refrigeration temperature (40°F); and b) its proven ability to prevent psychrotrophic development in raw milk. The organisms were isolated from an approved and widely used cheese starter culture (11). The culture is flash frozen in pellet form for easy handling; and, fast thawing; and, distribution in cold milk. The pellets are packaged in a half gallon paper carton with a pourable spout. One unit (1/2 gal.) inoculates up to 100,000 pounds of raw milk. Since the RMI culture is in pellet form, quantities of raw milk less than 100,000 pounds can be easily seeded.
Plant Trials

Several commercial trials of cheese manufacture were conducted over extended periods of time in which the RMI was used to inoculate raw milk to measure the differences in cheese yields between inoculated vs. uninoculated raw milk in storage holding tanks. Cheese yields were calculated by monitoring the total solids initially in the cheese vat vs. the total solids recovered in cheese, e.g., moisture free basis. The results of trials are shown in Table 9 (10). The differences of 0.138 and 0.125% increased yields from raw inoculated milk vs. the uninoculated milk are significant. The efficiency of solids recovered in trial one was greater with RMI than in trial two based upon the comparable differences of "Average % solids" between the inoculated vs. uninoculated milk in each trial. Other commercial trials have given similar results.

Cost Savings

Obviously, the total net savings one could expect is directly related to the percentage yield increase which is greatly influenced by the degree and magnitude of proteolysis by the psychrotrophic types and total numbers infecting a particular raw milk supply. Psychrotrophic contamination of raw milk does vary from day to day with given milk supplies, from milk shed to milk shed, and from plant to plant. The degree of contamination is unpredictable. And, until legal permission would be given to practice this technique at the farm/producer level, better control is difficult. Since psychrotrophic contamination is so unpredictable, some manufacturers look at this process as insurance. Even though we do not have a problem today there is no guarantee that we will not have a problem tomorrow.

Another benefit of adding lactic cultures to raw milk to activate LPS for controlling psychrotrophs to improve yields is the potential reduction of
degrades due to off-flavors, e.g., fruity, fermented, lipolytic, bitterness, etc. According to a survey by Broske, et al (1982), 3.2 percent of the degrades of cheddar cheese in Wisconsin was due to hydrolytic rancidity, which resulted in an annual loss of 2.3 million dollars. Quite often, hydrolytic off-flavors are directly related to the degree of psychrotrophic contamination in raw milk.

Personal communications from USDA graders of CCC held cheese have been positive in that cheese from certain plants have demonstrated a longer shelf life when it was manufactured from inoculated milk.

Some cheese manufacturers have also commented that they observed a stimulation of their starter culture activity to the point that some reduction in starter usage was possible.

**Legality**

The RMI process is approved by both the FDA and the USDA (20,16). Both have issued acceptable guidelines for cheese plants to practice this process. Copies of the USDA guidelines and the FDA letter of approval may be obtained upon request from Chr. Hansen's Laboratory, Inc. (10).

When inoculating raw milk (RMI), the basic approved procedures are: the cultures are added to the storage silo or tank at the plant. They are not approved for addition to the farm bulk tank, the farm bulk pickup transfer truck or at storage facilities located in a milk receiving or transfer station. *It may be added only at the plant where the milk is to be used directly for cheese manufacture.* The cultures must be approved and prepared in suitable facilities or the use of direct set cultures is permitted. The plant quality program and records must be adequate to ensure that the milk is of consistently good quality. Plant survey forms include the appropriate items for allowance. Both regulatory bodies stressed that pre-culturing shall not
be permitted as a substitute for good quality milk, sanitary plant equipment, or sanitary processing techniques.

**Control of Psychrotrophic Pathogen**

Another benefit from inoculating raw milk with an appropriate culture could be the control of specific psychrotrophic pathogens. In July at the CIFS&T (Canada Institute of Food Science and Technology) a report by Park and Stankiewicz (4) revealed that LPS was responsible for inactivating *Campylobacter jejuni*, a psychrotrophic pathogen, in raw milk. In their attempts to develop improved assay methods for determining the presence of *C. jejuni* when added to raw milk, it was necessary to add cysteine which inactivated LPS that was destroying *C. jejuni* when added to the milk system. The counts were reduced by four logs when stored for seven days, whereas only a reduction of 1.5 logs in count was observed when cysteine was added. No lactic organisms were added to produce hydrogen peroxide, nor did they report the presence of lactic bacteria in their samples. While cysteine prevented a major reduction in *C. jejuni* counts, it did not completely inactivate LPS as evidenced by a reduction of 1.5 logs with cysteine present. One could speculate that with adequate activation of LPS by sufficient levels of hydrogen peroxide (H₂O₂) e.g., by adding lactic culture, this particular psychrotroph could be controlled in inoculated raw milk.

*C. jejuni* is a gram negative pathogenic psychrotroph. Earlier, we referred to Reiter and Hamnulv (15) who reported LPS bactericidal activity against gram negative bacteria, e.g., *Eschericia*, *Salmonella* and *Pseudomonas* species. Pseudomonads are the predominant non-pathogen psychrotrophs found in refrigerated raw milk samples.

**Potentials of RMI**

A logical question could be: Has industry practice of refrigerating raw milk to very low temperatures (35°F), which prevent metabolic activity by
natural lactics for the production of sufficient quantities of hydrogen peroxide for LPS activation, permitted the increasing emergence of serious pathogenic psychrotrophs, e.g., *Listeria*, *Campylobacter*, *Yersinia* and *E. coli*? Would the inoculation of raw milk with lactic acid bacteria help to prevent the existence of these pathogens which can survive and grow at these low temperatures where the lactics cannot? Could we help ourselves by storing raw milk no colder than 40°F to insure LPS activity in the milk by the addition of sufficient number of lactics?

Returning to our opening statement "perhaps we have made our raw milk too clean microbiologically" has, we believe, some justification. Yes, the total plate counts of raw milk in general has been significantly reduced over the years because of tighter regulations. To insure that their raw milk meet regulatory standards for total mesophilic counts, the dairy farmers-producers have gradually lowered their farm bulk tank storage temperatures. By so doing, the mesophilic bacteria typically expressed by the Standard Plate Count method do not survive or are irreparably injured to where expression on agar is not possible without pre-enrichment. Yet, most all the psychrotrophs will survive very well and many will increase their numbers in raw milk stored at 35°F. *Listeria*, for example, has a growth temperature range from 2°C (35.6°F) to 45°C (113°F).

Much more raw milk is being routinely stored today at 5 to 10°F colder than it was some years ago. As stated earlier, storage temperatures of about 35°F will retard metabolic activity of natural lactics which is the "natural system" to activate LPS which has the ability to prevent some of our problems discussed here. Perhaps by our zeal to clean up our raw milk, e.g, less total mesophilic organisms, we may have permitted unconsciously and certainly unintentionally, the survival and/or growth of harmful psychrotrophic
pathogens as well as the non-pathogenic types which have caused economic losses and inferior quality in our dairy products.

Much research and development is still needed to elucidate the "reasons for", so that we can maximize our creativity innovations and practices that harmoniously fit into and compliment the "natural systems" which are already in existence.
REFERENCES


TABLE 1

TYPICAL MICROFLORA GRADE A RAW MILK

BULK TANK - MARCH 1983

TEMPERATURE - AT TIME TAKEN FROM BULK TANK -- 35°F

pH - 6.68  % T.A. - 0.13

CFU'S/ML

<table>
<thead>
<tr>
<th>DAY</th>
<th>TOTAL COUNT</th>
<th>SMA</th>
<th>PSYCHROTROPHS</th>
<th>COLI</th>
<th>MICROCOCCI</th>
<th>YEAST &amp; MOLD</th>
<th>LACTIC STREPS &amp; LACTOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>33,000</td>
<td>23,000</td>
<td>&gt;3,000</td>
<td>270 (--)</td>
<td>180</td>
<td>&lt;1,000 by DMC</td>
<td></td>
</tr>
</tbody>
</table>

BULK TANK - APRIL 1959

AVERAGE 4 SAMPLES

<table>
<thead>
<tr>
<th>DAY</th>
<th>TOTAL COUNT</th>
<th>SMA</th>
<th>PSYCHROTROPHS</th>
<th>COLI</th>
<th>MICROCOCCI</th>
<th>YEAST &amp; MOLD</th>
<th>LACTIC STREPS &amp; LACTOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>150,000</td>
<td>&lt;3,000</td>
<td>30,000</td>
<td>&lt;300</td>
<td>&lt;300</td>
<td>100,000</td>
<td></td>
</tr>
</tbody>
</table>

TEMPERATURE - AT TIME SAMPLES TAKEN -- 45-48°F

pH - 6.54  % T.A. - 0.15-0.16

CAN MILK - APRIL 1959

AVERAGE 4 SAMPLES

<table>
<thead>
<tr>
<th>DAY</th>
<th>TOTAL COUNT</th>
<th>SMA</th>
<th>PSYCHROTROPHS</th>
<th>COLI</th>
<th>MICROCOCCI</th>
<th>YEAST &amp; MOLD</th>
<th>LACTIC STREPS &amp; LACTOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>300,000</td>
<td>&lt;3,000</td>
<td>35,000</td>
<td>&lt;300</td>
<td>&lt;300</td>
<td>200,000</td>
<td></td>
</tr>
</tbody>
</table>

TEMPERATURE - 54°F

pH - 6.48  % T.A. - 0.18-0.19
<table>
<thead>
<tr>
<th>SAMPLE NO.</th>
<th>FRESH</th>
<th>24 HOURS</th>
<th>48 HOURS</th>
<th>72 HOURS</th>
<th>96 HOURS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5,100</td>
<td>5,000</td>
<td>103,000</td>
<td>420,000</td>
<td>1,750,000</td>
</tr>
<tr>
<td>2</td>
<td>2,800</td>
<td>3,400</td>
<td>5,100</td>
<td>7,500</td>
<td>8,600</td>
</tr>
<tr>
<td>3</td>
<td>46,000</td>
<td>87,000</td>
<td>184,000</td>
<td>470,000</td>
<td>2,490,000</td>
</tr>
<tr>
<td>4</td>
<td>340,000</td>
<td>510,000</td>
<td>2,400,000</td>
<td>4,600,000</td>
<td>11,000,000</td>
</tr>
</tbody>
</table>
**TABLE 3**

**ANALYSES ON RAW MILK HELD IN A STORAGE TANK AT 40°F**

<table>
<thead>
<tr>
<th>DAYS HELD</th>
<th>BACTERIAL COUNT</th>
<th>pH</th>
<th>FAT ACIDITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37,000</td>
<td>6.68</td>
<td>0.95</td>
</tr>
<tr>
<td>2</td>
<td>56,000</td>
<td>6.67</td>
<td>1.08</td>
</tr>
<tr>
<td>3</td>
<td>190,000</td>
<td>6.67</td>
<td>1.23</td>
</tr>
<tr>
<td>4</td>
<td>610,000</td>
<td>6.68</td>
<td>1.41</td>
</tr>
<tr>
<td>5</td>
<td>3,200,000</td>
<td>6.69</td>
<td>2.11</td>
</tr>
</tbody>
</table>
# TABLE 4

## ANALYSES ON RAW MILK HELD IN A STORAGE TANK AT 40°F

<table>
<thead>
<tr>
<th>DAYS HELD</th>
<th>BACTERIAL COUNT</th>
<th>pH</th>
<th>COAGULATION TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37,000</td>
<td>6.68</td>
<td>12.2 (Min.)</td>
</tr>
<tr>
<td>2</td>
<td>56,000</td>
<td>6.67</td>
<td>13.0</td>
</tr>
<tr>
<td>3</td>
<td>190,000</td>
<td>6.67</td>
<td>13.4</td>
</tr>
<tr>
<td>4</td>
<td>610,000</td>
<td>6.68</td>
<td>15.6</td>
</tr>
<tr>
<td>5</td>
<td>3,200,000</td>
<td>6.69</td>
<td>17.5</td>
</tr>
<tr>
<td>ORGANISM</td>
<td>ENZYME</td>
<td>TIME, MIN.</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>------------</td>
<td>------------</td>
<td></td>
</tr>
<tr>
<td>Pseudomonasfluorescens 22F</td>
<td>LIPASE</td>
<td>4.80</td>
<td></td>
</tr>
<tr>
<td>Ps. fragi 14-2</td>
<td>LIPASE</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>Ps. spp. 21B</td>
<td>PROTEINASE</td>
<td>250.0**</td>
<td></td>
</tr>
<tr>
<td>Ps. spp. MC 60</td>
<td>PROTEINASE</td>
<td>420.0**</td>
<td></td>
</tr>
</tbody>
</table>

(**) CALCULATED AND/OR EXTRAPOLATED

TABLE 6

YIELDS OF COTTAGE CHEESE

MANUFACTURED FROM MILK CONTAMINATED WITH PSYCHROTROPHS

AYLWARD, O'LEARY, AND LONGLOIS (1980)

LOST 2.5 TO 3.0% OF MAXIMUM YIELD PER DAY OF

STORAGE AT 5°C ON CULTURED COTTAGE CHEESE.

MOHAMED AND BASSETTE (1979)

LOST 2.6 TO 3.4% OF MAXIMUM YIELD PER DAY

STORAGE AT 8°C ON DIRECT ACID SET.
YIELDS OF CHEDDAR CHEESE

MANUFACTURED FROM MILK CONTAMINATED WITH PSYCHROTROPHS

NELSON AND MARSHALL (1977)

8 OF 9 STRAINS DID NOT AFFECT YIELD.

ONE ENTEROBACTER STRAIN DID AT $2 \times 10^5/\text{ML}$ AT

48 HOURS/5°C.

HICKS, O'LEARY AND BUCY (1977, 1978)

GRADE A MILK LOST 0.5% OF THE MAXIMUM YIELD PER

DAY OF STORAGE AT 5°C.

STOFER AND HICKS (1983)

MANUFACTURING GRADE MILK LOST 0.6% OF THE

MAXIMUM YIELD PER DAY OF STORAGE AT 5°C.
### TABLE 8

**INCREASE OF SOLUBLE NITROGEN IN CHEDDAR CHEESE WHEY.**

Milk was held at 40°F and a portion used to make cheese at 0, 3 and 5 days.

<table>
<thead>
<tr>
<th>Holding Time of Milk</th>
<th>Non-Protein Nitrogen Content of Whey</th>
<th>Bacterial Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Days</td>
<td>29.8 mg/100 g</td>
<td>32,000</td>
</tr>
<tr>
<td>3 Days</td>
<td>34.3</td>
<td>610,000</td>
</tr>
<tr>
<td>5 Days</td>
<td>42.1*</td>
<td>3,200,000</td>
</tr>
</tbody>
</table>

* THEORETICAL YIELD LOSS = c.a. 0.05%
<table>
<thead>
<tr>
<th></th>
<th>Trial 1</th>
<th>RMI</th>
<th>Untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Lbs. of Milk</strong></td>
<td>4,575,207</td>
<td>5,429,916</td>
<td></td>
</tr>
<tr>
<td><strong>Total Lbs. of Solids In</strong></td>
<td>542,212.31</td>
<td>653,520.56</td>
<td></td>
</tr>
<tr>
<td><strong>Total Lbs. of Solids Out</strong></td>
<td>271.410.84</td>
<td>322,303.78</td>
<td></td>
</tr>
<tr>
<td><strong>Lbs. of Cheese @ 36% Moisture</strong></td>
<td>424,079.46</td>
<td>503,599.66</td>
<td></td>
</tr>
<tr>
<td><strong>YIELD</strong></td>
<td>9.3855</td>
<td>9.2471</td>
<td>.1384</td>
</tr>
</tbody>
</table>

**TRIAL #2**

<table>
<thead>
<tr>
<th></th>
<th>Trial 2</th>
<th>RMI</th>
<th>Untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Lbs. of Milk</strong></td>
<td>2,700,000</td>
<td>3,375,000</td>
<td></td>
</tr>
<tr>
<td><strong>Total Lbs. of Solids In</strong></td>
<td>334,305.09</td>
<td>417,096</td>
<td></td>
</tr>
<tr>
<td><strong>Total Lbs. of Solids Out</strong></td>
<td>176,409.76</td>
<td>217,317.73</td>
<td></td>
</tr>
<tr>
<td><strong>Lbs. of Cheese @ 36% Moisture</strong></td>
<td>275,640.3</td>
<td>339,558.95</td>
<td></td>
</tr>
<tr>
<td><strong>YIELD</strong></td>
<td>9.8942</td>
<td>9.7692</td>
<td>.125</td>
</tr>
</tbody>
</table>
Effect of milk storage time on growth rate of psychrotrophic bacteria in Grade A (O---O) and manufacturing grade (●---●) milk. Milk was stored until coagulation occurred upon pasteurization.

STOFER AND HICKS (1983) CULT. DAIRY PROD. J. p.11-14
FIGURE 3

Effect of milk storage time on moisture content of cheddar cheese. Grade A milk (C—O). Manufacturing milk (●—●).

FIGURE 3

THE MODE OF ACTION OF LACTOPEROXIDASE SYSTEM

$\text{SCN}^- \quad + \quad \text{H}_2\text{O}$

$\Downarrow$

LP-Enzyme

$\downarrow$

$\text{O}_2\text{SCN}^-$

$\Downarrow$

$\text{CO}_2 + \text{NH}_4^+ + \text{SO}_4^{2-}$

(harmless oxidation end products)
**Figure 4**

**UNTREATED**

RAW MILK  →  LACTOPEROXIDASE SYSTEM (UNACTIVATED)  →  PSYCHROTROPHS  →  LIPASES  →  BUTTERFAT  →  CHEESE  →  OFF-FLAVOR NOTES

**RMI TREATED**

RAW MILK  →  ACTIVATED LACTOPEROXIDASE SYSTEM  →  INHIBITED PSYCHROTROPHS  →  PRESERVES  →  BUTTERFAT  →  CASEIN  →  CHEESE  →  IMPROVED FLAVOR  →  HIGHER YIELD
Appendix I


This is a compendium of published scientific literature on Bacteria and Rickettsiae Important in Human and Animal Health.

Recommended for all libraries as an excellent resource book.
TEACING CHEESE DEFECTS TO THEIR SOURCE
Robert L. Olsen, Ph.D.
Food Science Specialist
Department of Nutrition & Food Sciences
Utah State University

INTRODUCTION

Various methods of tracing defects in cheese to their source are practiced regularly in all cheese plants. Experts in cheese making are utilized. These people may come from either within or from outside the company. Efforts to eliminate defects are often unsuccessful. This is more often a result of the difficulty in assimilating the many factors involved in cheese making rather than inexperience or lack of knowledge of the experts.

EXPERT SYSTEMS

In order to more systematically approach a defect analysis problem, the use of artificial intelligence expert systems is being evaluated.

Artificial intelligence is the area of computer science concerned with designing intelligent computer systems. These are systems that exhibit the characteristics we associate with intelligence in human behavior, such as understanding, language, reasoning and solving problems.

Applications of AI which seek to replicate decision making by knowledgable and experienced humans are called expert systems. By transferring knowledge of human experts into a set of simple, interlinking rules, knowledge engineers are able to write programs which simulate an expert's reasoning.

Although serious efforts to create computers which mimic human reasoning have been underway for at least three decades, successful commercial applications are more recent. Those currently in use include the Prospector System, which aids in mineral exploration, Automated Cable Expert or ACE, which was developed to analyze telephone trouble reports, and Xcon, which is used for all of the configuring of DEC's vax computer systems. Novex interprets navigational data gathered in space shuttle flights. Early expert systems were MYCIN and
DENDRAL. MYCIN was designed to consult on problems of infectious disease diagnosis and therapy selection. DENDRAL helps in the determination of molecular structures by interpreting data from a mass spectrometer. Expert systems have also been developed for tasks such as planning experiments in molecular biology and designing organic syntheses (Davis, 1986).

In the food industry, Campbell Soup Company recently developed an expert system to preserve the expertise of a 44 year retiring employee. The system was designed to utilize his trouble shooting knowledge of large hydrostatic and rotary retorts (Tyson and Herrod, 1985).

Conventional computer programs are based on well established algorithms and focus on performance. You tell the computer what to do. With an expert system you tell the computer what it should know. This is done by separating the knowledge base from the inference engine. The knowledge base contains what the program should know. The inference engine confers with the knowledge base and processes the information. Multiple use can then be made of the knowledge base depending upon the problem at hand.

In developing an Expert System, numerous sessions are required involving the knowledge engineer and the expert. The knowledge engineer then develops the expert system structure that will best make use of the information.

Once developed, an expert system engages a user in a dialogue. This dialogue is similar to the type of conversation a person might have with an expert consultant. The computer is programmed to ask the user questions to detail the problem or situation. After collecting this information, the computer program combines the facts and rule-based logic to produce a solution to the user's problem (Ferrara et al., 1986).

In this study, three expert systems are being evaluated. Expert-Ease (Export Software International Ltd) is easy to learn and allows quick development. The user has little control over the inference strategy, however, and the tool cannot be interfaced to other software.

M1 (Teknowledge Inc.) is a sophisticated system which is more flexible than Expert-Ease. The form of inference is backward chaining,
from a hypothesis back toward observations that might support or
refute the hypothesis. Expert-Ease reasons forward from observations
to conclusions. M.1 has the ability to produce run-only end user
systems so work can be disseminated without purchasing numerous
copies of the expert system.

Insight II (Level Five Research) is a Pascal based program which
links to other programs, produces run-only end user versions, and is a
compiler-based system allowing it to run more rapidly (Ludvigsen et
al., 1986).

EXAMPLES

A sample from Expert-Ease is shown in Figure 1. The initial
inquiry and the final recommendations based on numerous responses to
questions presented by the system are given. The first response to
the question of body and texture or appearance is that the cheese is
open. Several subprograms will be called up depending on the
information given. The final response is a single recommendation which
is as long as the expert desires.

A comparable example is shown in Figure 2, using the Insight II
expert system tool. Insight II allows confidence intervals for user
responses which are not 100 percent true. Recommendations are then
expressed as either 100 percent or some figure less than 100 percent.

No example is shown for M.1.

REFERENCES


Expert system tools for civil engineering applications. In press.

Ferrera, J.M., Parry, J.D. and Lubke, M. 1986. M.1 and
Expert-Ease: Two expert system authoring tools for the
microcomputer. In press.

Food Engineering. 57(12):69.
Figure 1. Expert-Ease sample

EXPERT-EASE file: CHEDDAR3 41086 bytes left

What is the body and texture or appearance like? Is it ...

1. overfirm
2. curdy
3. molly
4. open
5. weak
6. crumbly
7. bleached

running CHEDDAR3
more -- press space to continue

EXPERT-EASE file: CHEDDAR3 41036 bytes left

Why don't you ...
... maintain the curd temperature between 85 F and 90 F.

Enter value 1..3
> 2
Figure 2. Insight II sample

CHEESE QUALITY ANALYSIS

Select what describes:

<table>
<thead>
<tr>
<th>Defect</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>open</td>
<td>weak</td>
</tr>
<tr>
<td></td>
<td>acid</td>
</tr>
<tr>
<td></td>
<td>bitter</td>
</tr>
<tr>
<td></td>
<td>unclean</td>
</tr>
<tr>
<td></td>
<td>whey</td>
</tr>
<tr>
<td></td>
<td>Corky</td>
</tr>
<tr>
<td></td>
<td>Crumbly</td>
</tr>
<tr>
<td></td>
<td>Curdy</td>
</tr>
</tbody>
</table>

A problem exists at cockpit
The specific causes are as follows:

<table>
<thead>
<tr>
<th>Issue</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Too rapid</td>
<td></td>
</tr>
<tr>
<td>Too high temperature</td>
<td>50</td>
</tr>
<tr>
<td>Too low temperature</td>
<td></td>
</tr>
</tbody>
</table>

A problem exists during Cheddaring
The specific causes are as follows:

<table>
<thead>
<tr>
<th>Issue</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blocks piled too high</td>
<td></td>
</tr>
<tr>
<td>Blocks too large</td>
<td></td>
</tr>
<tr>
<td>Not turning blocks often enough</td>
<td></td>
</tr>
<tr>
<td>Too low temperature</td>
<td>80</td>
</tr>
<tr>
<td>Blocks not piled high enough</td>
<td></td>
</tr>
<tr>
<td>Blocks too small</td>
<td></td>
</tr>
<tr>
<td>Too high temperature</td>
<td></td>
</tr>
</tbody>
</table>
CHEDDAR CHEESE: WHO SHOULD BE THE JUDGE?

by

Floyd W. Bodyfelt, Professor
Department of Food Science and Technology
Oregon State University, Corvallis

Who should be the judge of Cheddar cheese quality? The answer to this question is quite simply, "as many personnel as possible" that are involved in the process of converting milk into cheese, through distribution, sales and marketing steps.

Milk Quality—Prerequisite for Cheese Quality

Make no doubt about it, the sensory evaluation (or product judging) process must commence as early as the milk receiving stage of cheese production. An important premise in the dairy industry is, "that the quality of any finished dairy product can be no better than the quality of the raw materials that went into it." This is as true for Cheddar cheese as it is for any dairy food. Presently, many cheese processing plants in the U.S. are paying premiums for high solids (or high casein content) milk based on anticipated increases in cheese yield. Simultaneously, the overall quality attributes of milk should be given appropriate consideration for their marked impact on the quality characteristics of matured Cheddar cheese. The quality parameters of milk that have a bearing on cheese quality are: flavor, psychrotrophic bacteria content, somatic cell count, temperature history and age of milk, enzyme levels and freedom from microbial inhibitors (sanitizer and antibiotic residues).

Briefly, the following personnel have a most important role in determining the quality characteristics of the raw material (cheese milk) from which Cheddar cheese is made:

1. The milk producer (dairyman)
2. The milk hauler
3. The receiver of milk at the plant
4. Quality control personnel
5. The cheesemaker and crew members

The Cheesemaker—In Charge of Quality

As the milk is converted into curd, the cheesemaker (and assistants or team members) become more responsible or accountable for the quality characteristics of the cheese. In fact, alert cheesemakers can often project possible quality shortcomings long before laboratory analyses or "grading out" of the cheese may reveal defects. Based on this "fore-knowledge," the cheesemaker should attempt to "correct events" or make adjustments to "minimize the damage." Quite often remedial steps can be applied to minimize deterioration of quality in subsequent lots of cheese.
Cheddar Cheese—A Vehicle for Focus

Though this presentation will focus on Cheddar cheese, it would be interesting (time and space permitting) to discuss the quality parameters of some of the soft and semi-hard cheeses. The quality attributes of flavor, body and texture and color/appearance, Cheddar cheese serves as a convenient and familiar vehicle to examine the key sensory features that determine product quality and consumer acceptance.

Cheddar Cheese Score Cards

As early as 1915, the American Dairy Science Association (ADSA) proposed the first score card for assessing the sensory attributes of Cheddar cheese (Fig. 1). A score card is a tabulated list of the various quality parameters that help determine product acceptance. A range of numerical values are assigned each quality category (i.e. flavor, body/texture, color/appearance and finish). These numerical values are weighted among quality categories on the basis of their relative importance (see Fig. 1 and Table 1).

Obviously, the most important quality criterion for Cheddar cheese is flavor, which is assigned a score range of 1 to 10 (10 = "no criticism" or "no defect" and 1 = most unacceptable quality). Cheddar cheese, by this scoring format, that scores in the 7 to 10 range are generally considered quite acceptable. Usually, flavor scores in the 5 to 6 range are considered to exhibit "fair" flavor quality (improvement advised). A flavor score below 4 is indicative of a poor quality cheese (at least in the judgment of the evaluator[s]).

Cheese body and texture characteristics, as well as color and appearance, are assigned fewer points on the Cheddar cheese score card. Typically, a range of 1 to 5 points are assigned these two quality categories. A score of 5 indicates "no criticism; a score of 1 indicates severe body/texture or color/appearance defect(s) of the cheese.

It can be difficult to consider quality judgments of color and finish of Cheddar cheese unless the evaluator is examining a 40 lb block or 640 lb barrel of cheese. When paraffin-coated cheeses were commonplace, careful evaluation of the finish was an important factor, however, surface finish defects occur less often in contemporary cheese.

Sensory Evaluation of Cheddar Cheese

Table 1 is a set of recommended scores (scoring guideline) for various intensities of flavor and body/texture defects of Cheddar cheese. The flavor intensities are either slight, definite or pronounced. The more common flavor defects of Cheddar cheese will be examined one by one. Table 2 lists the more common flavor defects of cheddar cheese and their probable causes and remedial measures.
Acid off-flavor.—"High acid" is probably the most commonly encountered flavor defect in Cheddar cheese. Unless the high acid taste sensation exhibits a definite or pronounced intensity, it is not considered a serious defect. If a definite high acid taste note is detected at 60 days of age (ripening), it can be projected that the cheese might develop a bitter off-flavor after a few months of additional aging. If there is a definite or pronounced acid off-flavor evident in a young Cheddar cheese (i.e., less than 3 months), it is usually advisable to market that cheese immediately, since the acidity often becomes more intense with additional aging. Aging of a "high acid" cheese often leads to an intense bitter off-flavor, eventually. High acid and/or bitter off-flavors in young Cheddar cheese is often indicative of too rapid or excessive acid development in cheese milk or at critical points in the course of cheesemaking (especially at the time of whey draining).

Apparently, many consumers (to the industry's advantage) tend to mistakenly accept a combination of the high acid and/or bitter off-flavor for the sharp, aged or nutty-like flavor of Cheddar cheese. Most connoisseurs of fully matured Cheddar cheese seek a developed flavor which is distinctively nutty or filbert (hazel-nut)-like. Unfortunately, even in this scientific age and considering what we know about cheese technology, sufficient development of this desirable, distinct nutty flavor is not achieved in many lots of Cheddar cheese. More research is needed to facilitate the process of consistently achieving this nutty flavor in a larger proportion of the medium and aged Cheddar cheese manufactured in the United States.

Bitter.—"Bitterness" in Cheddar cheese is noted only by the sense of taste. Bitter off-flavors may occur in mild cheese, but are found more frequently in matured cheese. Certain lactic cultures, coagulating enzymes and salt levels have been implicated in the development of this defect. This taste defect has been observed to develop in cheese made from both excellent and poor quality milk. The bitter sensation is somewhat delayed in terms of initial perception, and the off-taste tends to persist for some time after sample expectoration.

Fruity/Fermented.—Fortunately, the "fruity" or "fermented" defect is practically a problem of the past. Occasionally, samples of Cheddar cheese appear that may manifest this defect, which are described as a fermented (vinegar) or fruity (pineapple) off-flavor. This defect was apparently caused by the use of certain Streptococcus lactis strains of cheese cultures. After the U.S. Cheddar cheese industry converted to Streptococcus cremoris cultures, the fermented/fruity defect was substantially reduced or eliminated.
Flat (Lacking Flavor).—A number of factors can lead to a "flat" or "lacks flavor" defect in Cheddar cheese. A young cheese at or under 60 days of age will generally exhibit flat or lacks flavor characteristics. Cheese graders or quality control personnel are generally concerned if a Cheddar cheese reaches four or six months of age, but still lacks any development of "Cheddar" flavor. Sometimes, the cheese may have been aged at too low temperature or there may have been insufficient acid development in manufacture, or the cheese may have been milled at too low of an acidity.

Heated.—The "heated" off-flavor in Cheddar cheese suggests that the milk was probably overheated during pasteurization. This off-flavor occurs rarely.

Moldy.—The "moldy" (musty) defect is often obvious by mere examination of the cheese for surface mold. A "musty-like" off-flavor migrates from the moldy surface toward the interior of the cheese.

Rancid.—The "rancid" defect (soapy, bitter, foul odor) results from the utilization of mishandled milk that has developed hydrolytic rancidity. Rancid off-flavor in milk is unpleasant enough, but the ten-fold concentration of milk solids in the course of making cheese results in a most intense and objectionable off-flavor, especially a foul aftertaste. Rancid or lipolytic off-flavor in Cheddar cheese is considered quite serious; it is certainly a problem that must be prevented.

Sulfide.—Occasionally, the protein of aged cheese breakdowns in a certain way (as the result of enzymatic activity) and releases several sulfur-based compounds. When a core sample of "sulfide" cheese is removed, a distinctive mercaptan or sulfhydryl aroma is observed. In a matured, high quality Cheddar cheese, part of the typical nutty or "Cheddar" flavor is a moderate level of the "sulfide" note. Cheddar cheese flavor is considered "out of balance" when an excessive sulfide character occurs. Some cheese graders describe the sulfide defect as "skunky cheese." Many consumers apparently prefer this particular flavor note in their cheese. So-called "sulfide cheese" tends to exhibit a "bite"; frequently an associated bitter and slight throat-burning sensation may accompany it. Frequently, a mealy-bodied defect is associated with a matured Cheddar cheese that exhibits the sulfide defect.

Unclean.—Usually, an unpleasant aftertaste in Cheddar cheese is the result of converting old or poor quality milk into cheese. As a rule, most food flavors should "cleanup" or not leave an aftertaste. In the instance of an "unclean" off-flavor, which occurs with some frequency in Cheddar cheese, there is a distinct, unpleasant aftertaste. Sometimes an unclean flavor defect may result from the retention of excess whey in the cheese (as the result of poor manufacturing practices). The retained whey may initially cause
the "whey-taint" flavor defect (often described as "dirty-sweet" off-flavor). With additional aging (medium or sharp), a more intense, unpleasant (unclean) aftertaste seems to develop. Certain amines and aldehydes that emerge from a particular form of protein degradation have been shown to be responsible for this more objectionable off-flavor in Cheddar cheese. Fresh, high quality cheese milk and control of the cheesemaking process minimize occurrence of the "unclean" off-flavor.

Whey taint.—"Whey taint" is a commonly encountered flavor defect in Cheddar cheese. It is assumed to be due to an atypical and delayed fermentation of the lactose within retained whey. This development is generally due to incomplete whey drainage in various stages of cheese manufacture, especially the cheddaring phase.

Yeasty.—Most fortunately, "yeasty" off-flavor is seldom encountered in Cheddar cheese. When it does occur, the yeasty defect is the result of poor packaging, wherein yeast colonies growing on the cheese surface produce aromas and migrate into the cheese. Yeasty is described as an earthy, alcohol-like off-flavor.

Balanced Component Theory of Cheddar Cheese Flavor

For Cheddar cheese of high quality, there is concern about hydrolysis of the major cheese components (i.e., protein, carbohydrates and milkfat). A number of years ago, Drs. Frank Kosikowski and G. Mocquot developed what has been generally referred to as the "balanced component theory" of Cheddar cheese flavor. This theory of flavor emphasizes having the "correct balance" or appropriate relative concentrations of several key chemical compounds within the volatile fraction of the cheese. Gas liquid chromatography (GLC) analysis of Cheddar cheese has shown that there are as many as 200 different compounds that may be involved in cheese flavor. However, flavor chemists believe that as few as 20 or less volatile compounds are pertinent to determination of the eventual flavor of Cheddar cheese. What appears critical is the relative proportions of the key chemical flavor compounds (see Table 3) in providing "balanced Cheddar flavor."

Body and Texture of Cheddar Cheese

The evaluator or cheese judge is concerned about body and texture, since the user (consumer) is looking for certain functional properties in the cheese (i.e., melting, grinding or slicing properties). Cheddar cheese continuously changes in body and texture characteristics within the ripening (aging) process due to changes in the protein. Casein components become more water soluble with additional ripening. Enzymatic activity hydrolyzes the casein. If a given cheese is aged long enough (more than 4 years), an ammonia-like odor may eventually occur. Cheese ripening is generally terminated before that point is reached. Within the ripening process, concern
about potential development of objectionable intensities of bitterness is a concern. If certain levels of peptides develop within the cheese, these compounds often account for bitter off-flavor.

Corky, Crumbly.—"Corky" and "crumbly" are quite similar body/texture defects, only the age of the cheese determines which defect has occurred. When Cheddar cheese is "worked" (tempered) and manipulated between the fingers, the cheese is supposed to be smooth and waxy (homogenous). High quality Cheddar cheese after proper tempering and manipulation should form an intact "marble" or cheese ball. Some cheese graders jokingly say "an evaluator should be able to enter a marble tournament with such a properly worked cheese sample," (if it has the appropriate pliability). On the other hand, if the sample crumbles this is considered a defect. In young cheese the friable curd is called "corky," in matured cheese the non-pliable cheese is called "crumbly." These features can be important when functional uses of the cheese (e.g., slicing, dicing, grinding, etc.) are considered.

Curdy.—The texture characteristic of a firm rubbery curd that is springy and noncohesive is referred to as "curdy." This property is usually noted in a young cheese (less than 1 or 2 months). The casein has not hydrolyzed to any extent at this early point in the development of curd to cheese. The "crude protein" or "green curd" characteristics prevail, since there are relatively few water soluble protein components at this point.

Gassy.—"Gassy" refers to the slits or small, symmetrical holes that exhibit a sheen on a cut surface of Cheddar cheese. Gas holes are the result of carbon dioxide formation by either starter culture bacteria or other (adventitious) microorganisms within the cheese. The lack of gas-holes (or slit-openness) or slits is primarily an aesthetic feature, though extreme gassiness is frequently associated with certain off-flavor defects (fruity/fermented) and may detract from the slicing characteristics of cheese.

Mealy.—A common characteristic of an older cheese is the texture defect "mealy." When such a cheese sample is tasted, masticated cheese pushed against the roof of the mouth will readily manifest distinct graininess (cornmeal-like). A slight to moderate degree of mealmess is quite often anticipated in a fully matured cheese, due to formation of several salt complexes with amino acids and/or lactic acid (e.g., calcium lactate, calcium tyrosinate, etc.).

Open.—The open defect of Cheddar cheese stems from mechanical openings, partially due to trapped air within the curd during pressing. The curd may have cooled too much prior to pressing (≤ 32°C [≤ 90°F]). This permits the milkfat to solidify, which results in curd particles not properly fusing together. In recent years it has often been customary, to pull a vacuum on the cheese.
when pressed; this action serves to pull a greater proportion of air out of the cheese and, hence, reduce openness. An excessive amount of large, mechanical openings can detract from slicing properties, as well as the desired appearance and workmanship of Cheddar cheese.

Pasty.—A more objectionable characteristic of "stickiness" or pasty is especially observed in unsalted Cheddar cheese. Occasionally, the pasty defect is noted in certain aged cheeses. When pasty cheese is hand "worked," the sample definitely tends to stick or adhere to the evaluator's thumb (resembles the tacky or sticky consistency of process cheese).

Short.—A core sample of pliable Cheddar cheese should bend about one-fourth to one-half way through an arc before it breaks apart. If the sample plug is bent a short distance (less than 1/4 arc) before fracture, this property of brittleness or lack of elasticity is called "short."

Weak.—By contrast, if an evaluator bends a 4 to 5 in long plug of cheese and can nearly make the terminal ends meet or touch (before fracture), the cheese "weakness" is often due to high moisture. This suggests better workmanship and control is needed in cheese manufacture.

Table 4 summarizes the common body and texture, color and appearance defects of Cheddar cheese, their probable causes and remedial measures. The rationale or basis for determination of the USDA Grades of Cheddar cheese are summarized in Table 5.

Packaging and Finish.—The cheese judge cannot discount the importance of the cheese package and the product "finish." The package and finish serve as a "silent salesman" for Cheddar cheese. The most important criteria are clean exterior surfaces and an attractive appearance. Many consumers criticize the cheese industry for lack of foresight in developing a package that can be resealed readily after it is opened. Obviously, the perfect cheese package has yet to be designed and implemented.

Summary and Conclusions

High quality Cheddar cheese is a product that has not been forced into maturity. When attempts are made to age Cheddar cheese at a more rapid rate (e.g., 55° or 60°F [13°-15°C]), problems of mold growth and certain off-flavors frequently occur. Patience is called for in cheese ripening. The industry standard for high quality Cheddar cheese is a full, balanced component (nutty) flavor; sharp, but not bitter. The cheese industry (and its consumers) are too often satisfied with substituting high acid or bitter flavor notes for the more desirable sharp, nutty flavor, in a fully matured cheese. The body of an aged Cheddar cheese should be slightly flaky (dry). Stated another way, the cheese should almost fall apart when it is "tickled by a knife."
If the cheese industry can produce Cheddar cheese that exhibits most of the aforementioned characteristics of a high quality cheese, then the manufacturer has a product that can proudly stand behind its brand name or label, whether it is the USDA's grade shield, or other "labels of distinction."

The sensory evaluation or judging of a given company's Cheddar cheese in an "informal competition" or comparison with cheese samples of competitor firms is an excellent way to assess the true "eating quality" of your product. Quality conscious manufacturers of cheese should constantly strive to determine the relative degree of excellence of their product. Indeed, cheese manufacturers and their personnel at several levels need to be the most critical judges of their own product. We highly recommend that cheese industry personnel at all levels practice the art of cheese judging at regular intervals.

A cheesemaker should never misplace his or her trier.
TABLE 1
RECOMMENDED SCORING GUIDE (A.D.S.A) FOR FLAVOR, 
BODY AND TEXTURE OF CHEDDAR CHEESE FOR DESIGNATED DEFECT 
INTENSITIES

<table>
<thead>
<tr>
<th>Flavor Criticisms</th>
<th>Intensity of Defect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slight b</td>
</tr>
<tr>
<td>High acid, sour</td>
<td>9</td>
</tr>
<tr>
<td>Bitter</td>
<td>9</td>
</tr>
<tr>
<td>Fermented, fruity</td>
<td>8</td>
</tr>
<tr>
<td>Flat, lacks flavor</td>
<td>9</td>
</tr>
<tr>
<td>Garlic, onion, weedy</td>
<td>6</td>
</tr>
<tr>
<td>Heated, cooked</td>
<td>9</td>
</tr>
<tr>
<td>Malty</td>
<td>8</td>
</tr>
<tr>
<td>Metallic</td>
<td>7</td>
</tr>
<tr>
<td>Moldy, musty</td>
<td>7</td>
</tr>
<tr>
<td>Rancid, lipase, putrid</td>
<td>6</td>
</tr>
<tr>
<td>Sulfide, skunky</td>
<td>9</td>
</tr>
<tr>
<td>Unclean, dirty</td>
<td>8</td>
</tr>
<tr>
<td>Whey-taint, sour-whey</td>
<td>8</td>
</tr>
<tr>
<td>Yeasty</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Body and Texture d Criticisms</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Corky, dry</td>
<td>4</td>
</tr>
<tr>
<td>Crumbly, friable</td>
<td>4</td>
</tr>
<tr>
<td>Curdy, rubbery</td>
<td>4</td>
</tr>
<tr>
<td>Fish eyes, slits, yeast holes</td>
<td>3</td>
</tr>
<tr>
<td>Gassy, pin holes</td>
<td>3</td>
</tr>
<tr>
<td>Greasy, salvy</td>
<td>3</td>
</tr>
<tr>
<td>Mealy, grainy</td>
<td>4</td>
</tr>
<tr>
<td>Open, mechanical holes</td>
<td>4</td>
</tr>
<tr>
<td>Pasty, sticky</td>
<td>3</td>
</tr>
<tr>
<td>Short, brittle, flaky</td>
<td>4</td>
</tr>
<tr>
<td>Sweet curd holes, Swiss eyes</td>
<td>4</td>
</tr>
<tr>
<td>Weak, soft, spongy</td>
<td>4</td>
</tr>
</tbody>
</table>

a "No criticism" for flavor merits a score of "10."

b Highest assignable score for a given defect for slight intensity.

c Lowest assignable score for a given defect for pronounced intensity.

d "No criticism" for body and texture merits a score of "5."
<table>
<thead>
<tr>
<th>Flavor Defect</th>
<th>Probable Causes</th>
<th>Remedial Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bitter</td>
<td>1. Excessive moisture</td>
<td>1. Use carefully selected cultures</td>
</tr>
<tr>
<td></td>
<td>2. Low salt level</td>
<td>2. Reduce amount of starter</td>
</tr>
<tr>
<td></td>
<td>3. Proteolytic starter culture strains</td>
<td>3. Monitor salting levels and method of adding</td>
</tr>
<tr>
<td></td>
<td>4. Microbial contaminants</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. Excessive acidity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6. Poor quality milk</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7. Plant sanitation problems</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. Upgrade milk quality</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5. Improve sanitation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6. Control acid and rate of development</td>
</tr>
<tr>
<td>High Acid (Sour)</td>
<td>1. Development of excessive lactic acid</td>
<td>1. Reduce ripening time</td>
</tr>
<tr>
<td></td>
<td>2. Excessive moisture</td>
<td>2. Reduce starter amount</td>
</tr>
<tr>
<td></td>
<td>3. Use of too much starter</td>
<td>3. Monitor milk acidity</td>
</tr>
<tr>
<td></td>
<td>4. Use of high acid milk</td>
<td>4. Cook to slightly higher temperature</td>
</tr>
<tr>
<td></td>
<td>5. Improper whey expulsion from curd</td>
<td>5. Follow a standardized procedure for cutting,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cooking, draining, cheddaring and salting steps</td>
</tr>
<tr>
<td></td>
<td>6. Low salt level</td>
<td></td>
</tr>
<tr>
<td>Flat (Lacks Flavor)</td>
<td>1. Lack of acid production</td>
<td>1. Check starter activity</td>
</tr>
<tr>
<td></td>
<td>2. Use of milk low in fat</td>
<td>2. Increase starter amount</td>
</tr>
<tr>
<td></td>
<td>3. Excessively high cooking temperature</td>
<td>3. Increase curing temperature</td>
</tr>
<tr>
<td></td>
<td>4. Use of too low curing temperature</td>
<td>4. Lengthen curing period</td>
</tr>
<tr>
<td></td>
<td>5. Too short curing period</td>
<td>5. Standardize cheese milk for fat content</td>
</tr>
<tr>
<td>Fruity/Fermented</td>
<td>1. Certain strains of \textit{S. lactis} or \textit{S. diacetylactis}</td>
<td>1. Eliminate lactic strains that produce ethanol</td>
</tr>
<tr>
<td></td>
<td>2. Low acidity</td>
<td>2. Monitor starter activity</td>
</tr>
<tr>
<td></td>
<td>3. Excessive moisture</td>
<td>3. Check salting procedures</td>
</tr>
<tr>
<td></td>
<td>4. Low salt level</td>
<td>4. Upgrade milk quality</td>
</tr>
<tr>
<td></td>
<td>5. Poor milk quality</td>
<td></td>
</tr>
<tr>
<td>Rancid (Soapy)</td>
<td>1. Milk lipase activity</td>
<td>1. Check cheese milk for rancid off-flavor</td>
</tr>
<tr>
<td></td>
<td>2. Microbial lipases from contaminants</td>
<td>2. Avoid excessive agitation, foaming and severe temperature</td>
</tr>
<tr>
<td></td>
<td>3. Accidental homogenization of raw milk</td>
<td>fluctuations</td>
</tr>
<tr>
<td></td>
<td>4. Late lactation or mastitic milk</td>
<td>3. Improve sanitation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. Monitor milk quality</td>
</tr>
</tbody>
</table>
### TABLE 2 (cont.)

<table>
<thead>
<tr>
<th>Problem</th>
<th>Causes</th>
<th>Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whey taint</td>
<td>1. Poor whey expulsion from curd</td>
<td>1. Standardize the Cheddaring process</td>
</tr>
<tr>
<td></td>
<td>2. Improper Cheddaring techniques</td>
<td>2. Constantly make sure expelled whey is free to drain away from Cheddaring curd</td>
</tr>
<tr>
<td></td>
<td>3. Failure to drain whey from piles of curd slabs (especially between pieces)</td>
<td>3. Wash curd with 32°C (90°F) water to remove excess whey</td>
</tr>
<tr>
<td>Unclean</td>
<td>1. Poor quality off-flavored or old milk</td>
<td>1. Upgrade milk quality</td>
</tr>
<tr>
<td></td>
<td>2. Unwanted microbial contaminants</td>
<td>2. Improve sanitation</td>
</tr>
<tr>
<td></td>
<td>3. Allowing off-flavored cheese to be &quot;aged&quot;</td>
<td>3. Market marginal quality cheese as mild</td>
</tr>
<tr>
<td></td>
<td>4. Improper technique of Cheddaring</td>
<td>4. Standardize the Cheddaring process</td>
</tr>
</tbody>
</table>

(Source: Chandan, R.C. 1980A)
### Table 3

The character of Cheddar cheese flavor based on the relative concentrations of free fatty acids and hydrogen sulfide

<table>
<thead>
<tr>
<th>Character</th>
<th>Intensity</th>
<th>F.F.A mm/g</th>
<th>H$_2$S mM/100g</th>
<th>F.F.A : H$_2$S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balanced</td>
<td>mild</td>
<td>10</td>
<td>0.7</td>
<td>14 : 1</td>
</tr>
<tr>
<td></td>
<td>sharp</td>
<td>28</td>
<td>2.0</td>
<td>14 : 1</td>
</tr>
<tr>
<td>Sulfide-like</td>
<td>mild</td>
<td>7</td>
<td>1.0</td>
<td>7 : 1</td>
</tr>
<tr>
<td>(Unclean)</td>
<td>sharp</td>
<td>21</td>
<td>3.0</td>
<td>7 : 1</td>
</tr>
<tr>
<td>Fatty acid-like</td>
<td>mild</td>
<td>11</td>
<td>0.4</td>
<td>28 : 1</td>
</tr>
<tr>
<td>(Fermented)</td>
<td>sharp</td>
<td>42</td>
<td>1.5</td>
<td>28 : 1</td>
</tr>
</tbody>
</table>

(From Kristoffersen 1963.)
### TABLE 4

**COMMON BODY AND TEXTURE, COLOR AND APPEARANCE DEFECTS OF CHEDDAR CHEESE, THEIR PROBABLE CAUSES AND REMEDIAL MEASURES**

<table>
<thead>
<tr>
<th>Body and Texture Defects</th>
<th>Probable Causes</th>
<th>Remedial Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corky, dry and hard</td>
<td>1. Lack of acid development</td>
<td>Follow standard or recommended procedures for cheese making</td>
</tr>
<tr>
<td></td>
<td>Excessive acid production and low moisture retention in cheese</td>
<td>1. Avoid ripening at higher temperature</td>
</tr>
<tr>
<td>Crumbly, mealy/grainy</td>
<td>Inadequate curing conditions</td>
<td>2. Control acid development and moisture level in curd</td>
</tr>
<tr>
<td>Curdy or rubbery</td>
<td>1. High moisture retained by curd</td>
<td>Control acid development in relation to time and temperature parameters</td>
</tr>
<tr>
<td>Pasty, sticky or wet</td>
<td>2. Excessive acid development</td>
<td></td>
</tr>
<tr>
<td>Weak or soft</td>
<td>1. Excessive fat content</td>
<td>1. Standardize fat in cheese milk</td>
</tr>
<tr>
<td></td>
<td>2. High moisture in cheese</td>
<td>2. Cook curd to desirable firmness (higher temperature, longer time)</td>
</tr>
<tr>
<td></td>
<td>3. Failure to develop &quot;body&quot; in cheese during cooking</td>
<td>3. Avoid piling curd slabs too high or too soon while cheddaring curd</td>
</tr>
</tbody>
</table>

**Color & Appearance Defects**

<table>
<thead>
<tr>
<th>Color-cut, bleached or faded (or dull looking), [portions or entire cheese surface]</th>
<th>1. Excessive acid development in the whey or at packing stage</th>
<th>1. Monitor acid development carefully</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2. Non-uniform moisture distribution in the cheese</td>
<td>2. Take precautions to insure consistent and uniform moisture retention in curd</td>
</tr>
<tr>
<td>Appearance</td>
<td>Cause</td>
<td>Recommendations</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>----------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Mottled appearance:</td>
<td>Irregularly shaped, light and dark areas on cheese surface</td>
<td>1. Avoid adding starter culture after color incorporation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Attempt to cut curd into uniform size particles</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Handle all curd carefully to avoid drying during matting, cheddaring or &quot;hold-overs&quot;</td>
</tr>
<tr>
<td>Seamy: shows light colored lines</td>
<td>Exudation of milkfat from curd pieces due to excessive forking, too</td>
<td>1. Wash &quot;greasy&quot; curd at 32°C (90°F) and thoroughly drain</td>
</tr>
<tr>
<td>around curd pieces</td>
<td>warm temperatures, and lack of salt dissolution</td>
<td>2. Avoid over-forking of the curd</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Allow all the salt to dissolve completely</td>
</tr>
<tr>
<td>White specks: granules or small</td>
<td>Generally, occurs in aged cheese. Derived from proteolysis and</td>
<td>1. Ripen cheese at higher temperature for shorter time.</td>
</tr>
<tr>
<td>hard mineral deposits</td>
<td>crystallization of calcium lactate/tyrosinate complex</td>
<td>2. Reduce levels of CaCl₂ added to cheese milk</td>
</tr>
<tr>
<td>Moldy appearance</td>
<td>Growth of mold on cheese surface</td>
<td>1. Insure airtight seals on cheese packages</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Avoid O₂ in the packages by vacuum, CO₂ or N₂ gas flushing</td>
</tr>
</tbody>
</table>

(Source: Chandan, R.C. 1980B)
<table>
<thead>
<tr>
<th>Grade</th>
<th>General description of medium cured to aged Cheddar</th>
<th>Approximate score or score range*</th>
<th>Grade</th>
<th>General description of medium cured to aged cheddar</th>
<th>Approximate score or score range*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>Flavor: Fine, highly pleasing, very slight feed flavor permitted.</td>
<td>93 or above</td>
<td>B</td>
<td>Flavor: May possess certain limited undesirable flavors according to age.</td>
<td>90 to 91</td>
</tr>
<tr>
<td></td>
<td>Body and Texture: Firm, solid, smooth, compact, close, translucent, few small mechanical, few sweet holes permitted, no gas holes.</td>
<td></td>
<td></td>
<td>Body and texture: Texture may be loose and open and have numerous sweet holes, scattered yeast and other scattered gas holes, pinny gas holes not permitted.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Color: Uniform, tiny white specks if aged and very slight seaminess permitted.</td>
<td></td>
<td></td>
<td>Color: May possess about the same defects as Grade A except to a greater degree.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Finish: Sound rind well protected and smooth, even shaped.</td>
<td></td>
<td></td>
<td>Finish: Rind sound, may be slightly weak, but free from soft spots, rind rot, cracks or openings, bandage may be uneven, wrinkled but sound, surface may be rough, unattractive, but have good protective coating; paraffin may be scaly or blistered; no indication that mold has entered the cheese; may be huffed, lopsided or have high edges.</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Flavor: Pleasing, may possess limited feed, acid or bitter flavor if aged.</td>
<td>92</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Body and texture: Reasonably solid, compact, close and translucent, few mechanical holes not large or connected, limited to two sweet holes per plug, no gas holes.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Flavor: May possess somewhat objectionable flavors and odors with certain increase tolerance according to age and degree of curing.</td>
<td>89</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Body and texture: May be loose with large connecting mechanical openings; have various gas holes and body defects with limitations varying with the degree of curing; must be sufficiently compact to permit drawing a full plug.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Color: May possess various defects, but not to the extent that the color is unattractive.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* These are the approximate numerical scores of each U.S. grade if scored by the scord-card system. The U.S. grades are reported in letter grades only.
FIG. 1. THE A.D.S.A. CONTEST CHEDDAR CHEESE SCORE CARD FOR SENSORY DEFECTS
WHEY
AND PERMEATE
UTILIZATION
Status of Whey & Whey Products Today

Dr. Warren S. Clark, Jr.
Executive Director
American Dairy Products Institute
130 North Franklin Street
Chicago, IL 60606

7th Biennial Cheese Industry Conference
Utah State University
August 26-28, 1986

The status of whey and whey products today directly relates to formal developmental efforts on behalf of the industry by its national trade association -- The Whey Products Institute -- and its new successor organization, The American Dairy Products Institute.

The Whey Products Institute was organized in 1971 as the national trade association of the whey products industry for the primary purposes of encouraging technological developments in support of whey processing, to develop viable markets for whey products, and to promote the use of these wholesome, nutritious and economical products as ingredients in a wide range of human food and animal feed products. For 15 years, these original goals have been pursued on behalf of whey processors while, at the same time, accepting the challenge of progress through change that will benefit not only whey processors, but, also, the entire dairy industry. On April 17, 1986, Members of the Whey Products Institute voted to merge WPI with the American Dry Milk Institute to form the American Dairy Products Institute.

For the past 15 years these two associations, while distinct and operating separately, each with its own policy-making Board and extensive committees, shared an administrative staff and office facilities. Gradually during that period it became more and more obvious that dry milk and dry whey processing were not only similar, but shared many of the same problems and concerns. With this realization, interest in combining the Institutes became an organized effort and, following nearly two years of study, evaluation and planning, merger of WPI and ADMI became a reality. We firmly believe that using the sound foundations from 61 years of successful operation of ADMI and 15 years experience in developing WPI, the American Dairy Products Institute has a bright future as it meets the existing and future challenges of the processed dairy products industry.

With the assistance of a series of slides presenting both whey and whey product production and utilization for over a decade -- a summary of much of these data being reflected in the materials distributed to you -- I will review first the development of the whey processing industry to where it is today; and, secondly, project what I believe lies ahead for whey processors on the basis of technological developments, supply-demand forecasts and other considerations.
Slides 1, 2 & 3 compare whey production in 1973-74, 1979-80 and 1984-85.


Slide 6 reflects the manufacture of modified whey products, and lactose, in the years 1975, 1980 and 1985.

All data reported on whey solids production and manufacture are based upon data furnished by the U.S. Department of Agriculture or calculated using USDA production data.

The next several slides present information on the use of whey solids, compiled by our association based upon industry reported end-uses. In 1975, WPI initiated the collection, compilation and dissemination of such end-use information -- a project that has been useful and meaningful to the industry (and others) throughout the 11 consecutive years of its publication. (Slide 7) In our 1976 Census of End-use -- reflecting the year 1975 -- approximately 70% of the USDA-reported whey solids processed were reflected in this report. In 1986 (reflecting calendar year 1985), 86% of the USDA-reported whey solids processed were covered by the report. In addition to the higher percentage of whey solids included in the current report, industry record keeping has improved significantly and the data are more reliable.

Slide 8 compares the utilization of concentrated sweet-type whey for human consumption in 1975 and 1985.

Slides 9 & 10 compare sweet-type dry whey human food uses in 1975 and 1985, while Slide 11 reflects that product's utilization in animal feeds.

Slide 12 presents data showing the use of acid-type whey solids for both human food and animal feed uses in 1975 and 1985.

I hope these visuals have been helpful in positioning the whey processing industry today in relation to where it was some 10-15 years ago. Next we need to project "where whey is going". Many years ago Little Miss Muffet contentedly -- until the untimely arrival of an Arachnid -- enjoyed curds and whey. But, what about now? Poor Little Miss Muffet most certainly would be confused for today she probably would find it difficult to recognize her whey -- and, in fact -- she might even lose her "whey".

Whey processing industry development began with fluid whey, which then was concentrated and dried. New developments added membrane technology to the existing traditional processes resulting in modified whey products such as reduced lactose and reduced minerals whey, whey protein concentrate, lactalbumin and lactose. Based upon her knowledge and experience, Little Miss Muffet quite likely wouldn't recognize these as whey products.

We've quickly transgressed from yesterday through today as far as whey processing is concerned -- but, what about the future? I believe that whey solids will continue to be used effectively and profitably in both human foods and animal feeds. Current emphasis is toward human food utilization, because of economic returns, and I believe the trend will continue in this direction.
Second, notwithstanding a multitude of short-term uncertainties, I believe that total U.S. milk production will decrease in the future. Given that the first priority of milk use is as Class I utilization, this will mean less milk for manufacturing -- to be divided among processors of cheese-whey and butter-nonfat dry milk. With lower volumes of milk and whey available, the supply-demand situation will improve markedly and product prices increase -- to a point where it will be comfortably - (rather than un- or marginally-) profitable to regularly process whey solids.

Third, the trend already in existence in whey processing will spread. Reference here is to fractionation with the various separated components of manufacturing milk selected and formulated to give the specific product desired for ingredient purposes. Accordingly, "blends" or blended products will become the "name of the game". These blends will not be limited to those containing only dairy ingredients, but will reflect a broad range of combinations with grain proteins and vegetable fats.

Current consumer preferences regarding food intake are for more protein and fiber, no significant change in carbohydrates, but with continued decreases in fat. Certainly, whey and the various whey components fit this need pattern very well.

At one time, the future for whey appeared to depend upon teaching cows to drink it. They do so, and with very positive end results both in terms of body growth and milk production. Accordingly, this is one alternative for future whey utilization and the extent of practice will depend heavily on supply volume and location, as well as on the viable alternatives for processing.

In considering whey handling and utilization in the future, one needs to consider where whey volumes will be originating. This will depend heavily on where milk is produced. Using a map of the U.S., circles can be drawn that represent future possible high concentration milk production areas -- based upon established production, production efficiencies, and/or consumption demand. These cover: 1.) Washington-Oregon-Idaho; 2.) Southern California-east; 3.) Wisconsin-Minnesota-Iowa; 4.) Northeast; and, 5.) Southeast. Primary supplies of whey from these areas certainly will present industry challenges -- and, with them, opportunities.

Current institute efforts are focused heavily on whey permeates, lactose, and increasing the utilization for all whey products. Increased utilization, hopefully, will not be in substitution for other dairy solids now in use. Among food products holding greatest potential for utilizing whey solids are beverage drinks, snack foods and a multitude of natural and health foods. Increased utilization, however, can only be accomplished through extensive research in the area of product development -- a direct responsibility of processors.

I believe that the strongest demand in the future will be for value added whey products (modified wheys), as well as for components derived from milk or whey that will be used in preparing any number of bioengineered foods.
In summary, the future for whey (processing and utilization) will be limited only by lack of a belief in the future of the industry and lack of foresight to properly prepare for it. Whey processors will not be "ultra-filtered" out of existence. An active whey processing segment will add strength and stability to the entire dairy industry.

* - * - *

- 4 -
## TABLE I.

### Whey & Whey Solids - Availability, Utilization*

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid Whey</td>
<td>28.7</td>
<td>29.9</td>
<td>37.4</td>
<td>39.5</td>
<td>45.7</td>
<td>50.9</td>
</tr>
<tr>
<td>Sweet-type Whey</td>
<td>24.1</td>
<td>25.8</td>
<td>33.4</td>
<td>35.5</td>
<td>42.1</td>
<td>45.1</td>
</tr>
<tr>
<td>Acid-type Whey</td>
<td>4.6</td>
<td>4.1</td>
<td>4.0</td>
<td>4.0</td>
<td>3.6</td>
<td>5.8</td>
</tr>
<tr>
<td>Whey solids equivalent</td>
<td>1.9</td>
<td>1.9</td>
<td>2.4</td>
<td>2.6</td>
<td>3.0</td>
<td>3.3</td>
</tr>
</tbody>
</table>

**Whey solids further processed**

<table>
<thead>
<tr>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td>Pounds</td>
<td>1.0</td>
<td>1.1</td>
<td>1.3</td>
<td>1.3</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Percent of available</td>
<td>55.0%</td>
<td>56.5%</td>
<td>52.9%</td>
<td>51.6%</td>
<td>52.1%</td>
<td>46.6%</td>
</tr>
</tbody>
</table>

*All figures in billions of pounds

## TABLE II.

### Dry Whey Production - Intended Utilization*

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Dry Whey Production</th>
<th>Human Food Use</th>
<th>Animal Feed Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>1960</td>
<td>277</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1965</td>
<td>404</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1970</td>
<td>621</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1975**</td>
<td>595</td>
<td>438 (73.6%)</td>
<td>157 (26.4%)</td>
</tr>
<tr>
<td>1980**</td>
<td>690</td>
<td>535 (77.5%)</td>
<td>155 (22.5%)</td>
</tr>
<tr>
<td>1985**</td>
<td>950</td>
<td>776 (81.7%)</td>
<td>174 (18.3%)</td>
</tr>
</tbody>
</table>

*Millions of pounds

**Excludes production of modified dry whey products
## Estimated U.S. Fluid Whey and Whey Solids Production (by type) and Resulting Quantity of Whey Solids Further Processed

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sweet-type Whey Production</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese Production</td>
<td>2,605</td>
<td>2,685</td>
<td>2,973</td>
<td>2,811</td>
<td>3,320</td>
<td>3,359</td>
<td>3,520</td>
<td>3,715</td>
<td>3,984</td>
<td>4,278</td>
<td>4,560</td>
<td>4,819</td>
<td>4,874</td>
<td>5,009</td>
</tr>
<tr>
<td>Calculated Whey Solids</td>
<td>1,524</td>
<td>1,371</td>
<td>1,718</td>
<td>1,665</td>
<td>1,942</td>
<td>1,965</td>
<td>2,059</td>
<td>2,173</td>
<td>2,331</td>
<td>2,502</td>
<td>2,656</td>
<td>2,819</td>
<td>2,734</td>
<td>2,930</td>
</tr>
<tr>
<td><strong>Acid-type Whey Production</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cottage Cheese Production</td>
<td>2,605</td>
<td>2,685</td>
<td>2,973</td>
<td>2,811</td>
<td>3,320</td>
<td>3,359</td>
<td>3,520</td>
<td>3,715</td>
<td>3,984</td>
<td>4,278</td>
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<td>Calculated Whey Solids</td>
<td>1,524</td>
<td>1,371</td>
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<td>1,942</td>
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<td>2,059</td>
<td>2,173</td>
<td>2,331</td>
<td>2,502</td>
<td>2,656</td>
<td>2,819</td>
<td>2,734</td>
<td>2,930</td>
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<tr>
<td><strong>Total Whey Production (fluid basis):</strong></td>
<td>28,149</td>
<td>29,724</td>
<td>30,573</td>
<td>29,505</td>
<td>34,166</td>
<td>34,335</td>
<td>35,908</td>
<td>60,619</td>
<td>39,858</td>
<td>42,890</td>
<td>43,834</td>
<td>47,079</td>
<td>45,686</td>
<td>50,871</td>
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<tr>
<td><strong>Total Whey Production (solids basis):</strong></td>
<td>1,830</td>
<td>1,958</td>
<td>1,987</td>
<td>1,918</td>
<td>2,210</td>
<td>2,232</td>
<td>2,332</td>
<td>2,432</td>
<td>2,391</td>
<td>2,725</td>
<td>2,901</td>
<td>3,060</td>
<td>2,969</td>
<td>3,306</td>
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<td>Whey Solids Further Processed:</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>A-Concentrated Whey Solids</td>
<td>70</td>
<td>70</td>
<td>61</td>
<td>88</td>
<td>146</td>
<td>145</td>
<td>144</td>
<td>100</td>
<td>86</td>
<td>113</td>
<td>142</td>
<td>136</td>
<td>130</td>
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<tr>
<td>B-Dry Whey</td>
<td></td>
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<tr>
<td>- Human Food</td>
<td>377</td>
<td>384</td>
<td>453</td>
<td>439</td>
<td>480</td>
<td>473</td>
<td>515</td>
<td>529</td>
<td>524</td>
<td>594</td>
<td>611</td>
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<td>736</td>
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<tr>
<td>- Animal Feed</td>
<td>385</td>
<td>389</td>
<td>399</td>
<td>157</td>
<td>182</td>
<td>155</td>
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<td>184</td>
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<td>C-Modified Dry Whey Products</td>
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<tr>
<td>- Reduced Lactose Whey</td>
<td>162</td>
<td>128</td>
<td>144</td>
<td>166</td>
<td>137</td>
<td>158</td>
<td>140</td>
<td>132</td>
<td>86</td>
<td>60</td>
<td>97</td>
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<td>- Reduced Minerals Whey</td>
<td>22</td>
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<td>- Whey Protein Concentrate</td>
<td>8</td>
<td>10</td>
<td>11</td>
<td>12</td>
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<tr>
<td>D-Whey Solids in Wet Blends</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>E-Whey Solids Utilized for Lactose</td>
<td>141</td>
<td>184</td>
<td>210</td>
<td>222</td>
<td>222</td>
<td>222</td>
<td>222</td>
<td>222</td>
<td>222</td>
<td>222</td>
<td>222</td>
<td>222</td>
<td>222</td>
<td>222</td>
</tr>
<tr>
<td><strong>Total Whey Solids Further Processed (A+B+C+D+E):</strong></td>
<td>923</td>
<td>1,027</td>
<td>1,123</td>
<td>1,159</td>
<td>1,214</td>
<td>1,194</td>
<td>1,339</td>
<td>1,287</td>
<td>1,337</td>
<td>1,486</td>
<td>1,560</td>
<td>1,567</td>
<td>1,546</td>
<td>1,522</td>
</tr>
</tbody>
</table>

| Total Whey Solids Further Processed as % of Total Whey Production (solids basis): | 53.7% | 59.07% | 59.53% | 60.42% | 58.78% | 57.55% | 57.25% | 56.92% | 56.65% | 56.07% | 51.12% | 51.02% | 52.12% | 46.65% |

1 Revised
2 Preliminary: pending revision
3 Crop Reporting Board, EFO, 1973-1981
4 Cheese production: approximately 9 lb/lb cheese produced (except Cottage)
5 Concentrated Whey production: approximately 6 lb/lb Cottage cheese produced
6 Average total solids content of concentrated whey: 43%
7 Data not available
8 Reduced Lactose and Reduced Minerals Whey combined to avoid disclosure of individual plant operations
9 Reported as Partially Delactosed/Decominalized through 1981
10 Approximately 1.6 lb whey solids utilized /1 lb lactose produced

(0836)
1. **Introduction**

It will be noted that the title assigned is a question, and it is presumed that the conference organizers expect this paper to give an answer to the question. It is, however, virtually impossible to give a simple answer to such a question, to meet the differing circumstances of cheese producers throughout the U.S. Nevertheless, we will try to set out the basic facts, so that each of you may be able to answer the question for yourself.

First of all, what sort of alcohol does the question envisage? The alcohol produced from whey is ethanol (otherwise referred to as ethyl alcohol), and it may be used for beverage purposes, (as vodka, or in the preparation of gin or cream liqueurs, etc.), or it may be used as industrial alcohol, for a wide range of applications from vinegar making to blood fractionation, or it may be used as fuel alcohol for blending with gasoline, to serve as a gasoline extender and octane booster. The economics for the three different types of alcohol will vary with the location, but in my experience with the "Carbery Whey-Alcohol Process", which was developed by Carbery Milk Products of County Cork, Ireland, I have been involved in producing all three basic types of alcohol from whey, in locations as far apart as New Zealand, California, and Ireland, and consider it is highly probable that one of the three types of alcohol will fit your particular set of circumstances.

It should be added that while we may generally refer to the whey from cheese production, the Carbery Whey-Alcohol Process has been applied equally effectively to acid-casein whey.

2. **Whey utilization or disposal**

In the manufacture of cheese, approximately 90% of the original milk volume is left as a liquid residue known as 'whey'. Thus for every pound of cheese produced, there is a residue of about 1.1 U.S. gallons of whey. As indicated in Table 1, whey normally contains about 0.6% protein, 0.6% ash (salts), and about 4.6% lactose or 'milk sugar'.

In the past, whey was simply fed to pigs, or run to waste, presenting pollution problems in rivers and streams around the cheese factories. In recent decades, however, with the trend towards greater centralization of cheese production facilities, the volumes of whey available began to exceed the requirements of the pig populations within reasonable trucking distance of the cheese factories, so that the disposal of whey for pig feed was no longer feasible.
Whey has a fairly high biological oxygen demand (B.O.D.) of about 350,000 ppm, so that the pollution effect of a moderately large centralized factory producing 75 U.S. tons of cheese per day and 165,000 U.S. gallons of whey would be the equivalent of the sewage effluent of a city of a population of 290,000 people. Obviously with the recently increased awareness of the need for protection of the environment, discharge of volumes of whey of this magnitude into our rivers would be completely unacceptable. On the other hand, the simple alternative of building and operating a sewage treatment plant of the necessary size would make the overall costs of cheese production prohibitive.

For several years, many cheese plants have been drying their whey to produce a whey powder, for sale mainly as an animal feed ingredient. The price of this commodity has varied along with the price of other animal feed proteins, although the costs of its production have risen sharply along with rising oil costs, due to the energy-intensive nature of the drying process. Thus, the economics of whey powder production have become very uncertain in recent years, and whey drying has been looked upon as a waste-disposal procedure rather than a route to a profitable by-product.

With the advent of ultrafiltration systems, it has become possible to remove the proteins from the whey in an undenatured state, to give a product ranging from 30-80% protein, which is of very high quality and value, being suitable for direct human consumption. The removal of the protein reduces the volume of the whey by about 15%, and reduces the total B.O.D. loading by about 20%, but still leaves the problem of the disposal of large quantities of ultrafiltration permeate.

The permeate contains most of the lactose and salts originally present in the whey (Table 1). It may be dried to produce a whey permeate powder, but this tends to be of limited value, due to its relatively high salt content.

Carbery Milk Products, Ltd., of Ballineen, Co. Cork, Ireland, has operated a large cheddar cheese plant beside a famous salmon-fishing river in West Cork since 1968, and has had to contend with the problems of whey utilization since its inception. At first, they dried the whey to a powder, and later they installed an ultrafiltration system for the production of whey protein concentrate.

When the Company was faced in 1976 with the problem of utilization or disposal of the whey ultrafiltration permeate, they examined the various options. These were (a) drying it as a permeate powder, (b) demineralizing it and drying it for the production of lactose, (c) anaerobic digestion for methane production, and (d) fermentation of the permeate for ethanol production. In the case of the last option the Company had the advantage of being a subsidiary of the London-based conglomerate Grand Metropolitan Company. Apart from owning many famous hotels around the world, including the 'Intercontinental' chain, "Grand Met" also owns International Distillers & Vintners, Ltd., (which is otherwise known as 'Gilbeys' and which holds the Smirnoff vodka production franchise for Britain, Ireland, and many other countries,) and the major British beer brewing company Watney, Mann, & Truman, Ltd. By pooling the research and development resources of Carbery with Gilbeys and Watneys, a study was made of the potential for ethanol production from whey on a commercial basis.

3. Development of the Carbery Process for whey-alcohol production

A considerable amount of experimentation was carried out in the Company's laboratories in England and Ireland in 1976 to discover the most suitable yeast strain for the fermentation of whey lactose, and to establish the optimum fer-
mentionation conditions. Fermentation and distillation trials were also carried out to examine the suitability of whey as a feedstock for beverage ethanol production.

(The possibility of producing fuel ethanol did not exist in Ireland, as although a government-controlled company had been set up in 1936 to produce fuel ethanol and has been in continuous operation since that date, the legislation requiring the oil companies to purchase its production did not extend to cover ethanol produced by private companies.)

After a year of concentrated effort it was concluded that the project would be commercially viable if the ethanol yields obtained in the laboratory trials could be achieved when the process was scaled-up to a full-size plant operation.

The decision to build the full-scale plant to produce ethanol from whey permeate at Carbery was taken early in 1977 and the project was treated with such a degree of urgency that it was successfully completed and in operation by April 1978. This accomplishment involved a considerable amount of planning and close cooperation between designers, equipment suppliers and fabricators, and the construction and plant personnel. It involved the design and construction of fermentation and distillation facilities, together with a treatment plant to handle the large volume of effluent.

From the initial daily volume of 140,000 U.S. gallons of whey permeate, the expected load, including normal effluent generated by equipment washing, was 9,000 lbs. of B.O.D. in a total effluent of about 200,000 U.S. gallons. Samples of the effluent from the laboratory trials were sent to treatment plant suppliers for treatability studies, and an extended aeration system with horizontal aerators was eventually selected. The treated effluent was required to meet international standards for final discharge into the nearby Bandon River which is noted for its salmon fishing.

The fermentation equipment consists of six fermentors of about 44,000 U.S. gallons working capacity, operated on a batch fermentation system, together with yeast propagation and storage tanks. A centrifuging system for separating and concentrating yeast was also installed. From the fermentors, the fermented permeate is pumped to the stills via holding tanks known as 'beer wells'.

As the Company wished to produce a very high quality neutral spirit of vodka standard, while nothing was known about the congeneric constituents which would have to be removed from the whey permeate ethanol to achieve this, (apart from some information obtained in pilot plant distillation trials), the distillation system was designed to be as versatile as possible. Consisting of six columns, it was extremely sophisticated and included numerous special features and accessories which were added to be able to deal with almost any conceivable problem which might be encountered. Thus, it was essentially a full-scale pilot plant.

The distillation system is carefully controlled, and the ethanol product is examined regularly by the operators and by an experienced taste panel to ensure that it is as clean and odor-free as possible, to meet the requirements of the most discriminating producers of vodka and other beverages. Gas chromatography is also used as a back-up to the organoleptic testing to assist in the control of the system, but cannot completely replace the human nose.

After an initial commissioning and development period of tuning-up the distillation system, an ethanol product was obtained which was completely free of odors or tastes characteristic of the raw material. The product quality is such that it is now used in other ethanol plants using grain or molasses feedstocks, as an organoleptic 'standard', or ideal to be aimed at.

The product is also required to meet the stringent European Community chemical standards, and is analyzed in the special ethanol plant laboratory...
which is also equipped with all the necessary systems for pure culturing of the special Carbery yeast strain and for chemical and microbiological monitoring of the process.

The venture has been considered to be an outstanding success and has achieved ethanol yields in excess of 85% of the theoretically attainable figures.

The operation is energy-intensive as the lactose content of whey permeate only averages about 4.7% to give an ethanol content of about 2.59% in the beer feed to the stills.

To reduce the unit cost of energy, experimental work was carried out on the concentration of the lactose prior to fermentation. It was found that lactose levels in excess of 10% can be fermented without significant losses in efficiency. Whilst it has not been considered economically advantageous to concentrate the permeate produced at Carbery before fermentation, the studies have had a significant impact on plant capacity in that whey permeate is now purchased from other cheese plants where it is concentrated to about 25% lactose, (to minimize transport costs), and it is mixed with the Carbery permeate to give an increased lactose level and an enhanced ethanol yield.

The total cost of the project in 1978 was about $1.45 million for plant and $0.96 million for buildings (at 1986 rates of exchange). A total of fifteen people are directly employed in the plant. The operation is energy-intensive, although this has become relatively less important as coal-fired boilers have now been installed at Carbery and have cut the cost of steam substantially. A breakdown of relative costs is given in Table 2.

Another major cost factor in addition to energy and labour is chemicals. These are used mainly in effluent treatment. A methane digestion plant has been installed to pretreat the increasing volumes of stillage effluent (coming from both increased purchases of concentrated permeate and increased cheese-plant output), and it has reduced the chemical usage as well as producing gas which is used as a supplementary source of energy.

To date, the Carbery whey-ethanol process has been used under license for two plants in New Zealand, owned by the New Zealand Co-operative Dairy Company, and one plant in California, owned by the Golden Cheese Company of California. The first plant in New Zealand, at Reporoa was commissioned in 1980 and is of a similar size to the Carbery plant. It involves a similar batch fermentation system and uses a stream-lined version of the distillation system, which eliminates some of the features which Carbery's experience had proved unnecessary.

It produces a similar high-quality spirit for vodka and for wine fortification. The plant also has an anhydrous ethanol production unit which satisfies the nation's requirements for anhydrous ethanol, including supplies to the National Alcohol Fuels Commission for road trials of ethanol-fueled vehicles.

The second plant in New Zealand at Tirau was commissioned in 1981, and produces approximately 2.5 times the output of the Carbery or Reporoa plants. It uses a continuous fermentation system which reduces labour requirements. It has a simple three-column distillation system for the production of industrial ethanol, and the plant's operation has turned the country from an importer of industrial ethanol to a net exporter.

Last year, in Southern California, under a construction and management contract, Carbery personnel commissioned one of the nation's largest cheese factories. It incorporates a whey-ethanol plant producing approximately 2.2 million U.S. gallons of anhydrous alcohol, which is sold for blending with gasoline on the Los Angeles market.
TABLE I

Constituents of Whey and Whey Permeate

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Whey</th>
<th>Whey Permeate</th>
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</thead>
<tbody>
<tr>
<td>Whey Proteins</td>
<td>0.60%</td>
<td>0.03%</td>
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<tr>
<td>Non Protein N</td>
<td>0.23%</td>
<td>0.23%</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.60%</td>
<td>4.70%</td>
</tr>
<tr>
<td>Ash</td>
<td>0.60%</td>
<td>0.65%</td>
</tr>
<tr>
<td>Fat</td>
<td>0.45%</td>
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TABLE II

Process Costs

<table>
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<th>% of Variable Costs</th>
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<td>Fuel &amp; Power</td>
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<td>Labour</td>
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<tr>
<td>Repairs</td>
</tr>
<tr>
<td>Chemicals</td>
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<td>Administration</td>
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</table>
4. **Choice of type of alcohol to produce**

The selection of type of alcohol to be produced from whey depends largely on the location and the potential outlets available.

(a) **Beverage alcohol.** The beverage alcohol market is the most stable, price-wise, although the demand is declining gradually. Prices for neutral spirit suitable for vodka or cream liqueur making are currently quoted at around $0.72 per proof gallon, which is equivalent to about $1.38 per U.S. gallon at 192 proof, which would be the normal production strength. (It should be noted that U.S. proof readings are double the percentage of alcohol by volume. Also, a "proof gallon" is a U.S. gallon of alcohol at 100 proof, (50% alcohol by volume), or it is the equivalent at other proofs, such as 0.5 U.S. gallons at 200 proof (100% alcohol by volume).

It is understood that in Wisconsin vodka and other beverages made from whey alcohol are exempt from certain state taxes, so that the selling price of whey neutral spirits might be expected to be somewhat higher there than the prices applicable elsewhere.

The beverage market, (apart from 'own-brand' bottlers), tends to be very conservative, and so it can be very difficult to break into the market, unless, for example, you already happen to be supplying cream to a cream liqueur producer, or have other established contacts. It should also be mentioned that U.S. federal law requires that liquor bottle labels denote the feedstock used for alcohol production if it is not grain, so that whey-alcohol is not freely interchangeable with grain alcohol for the production of major brands of beverages.

The production of neutral spirit requires some fairly sophisticated distillation equipment and controls, as the alcohol has to be carefully purified to remove all the undesirable 'congeners', or impurities which are formed in the fermentation process, and consequently requires a high degree of supervision.

(b) **Industrial alcohol.** There are various types and qualities of industrial alcohol, depending on the end user's requirements. The lowest quality may be 190 proof, and may have most of the congenic impurities still present. It would be produced on a simple two or three-column system without elaborate controls. Higher grades of industrial alcohol can be as pure as beverage neutral spirits and may be produced on sophisticated five-column units, or even seven-column units if it has to be dehydrated to be sold at 200 proof as 'anhydrous' or 'absolute' alcohol.

In normal distillation processes, it is impossible to concentrate alcohol beyond about 194 proof, (or 97% alcohol by volume), as an "azeotrope" or constant-boiling-mixture is formed at that point. In practice, the energy requirements for alcohol distillation increase disproportionately above about 185 proof, so that 190 proof is the normal practical limit for low grades of industrial alcohol, and 192 proof for higher grades.

In order to overcome the "azeotropic barrier", it is necessary to resort to special techniques to dehydrate alcohol, which involve the use of additional equipment. "Entrainers" such as benzene or cyclohexane or "extractants" such as glycerine, ethylene glycol, or salts may be used to remove the remainder of the water. Alternatively, one may use molecular sieve units to remove the water. They act like a water softener or demineralizer, in that the aqueous alcohol is passed through a cylinder containing the molecular sieve material which absorbs the water. When saturated with water, the material is regenerated and then re-used. Molecular sieves are becoming increasingly popu-
lar in that they are simple to operate and do not affect the composition of the alcohol, and do not leave any traces of undesirable compounds such as benzene.

Prices for industrial alcohol vary with the quality and location. In the past year the industrial alcohol market has been depressed by imports from a new 100 million gallon per year plant which recently came on stream in Saudi Arabia, where surplus natural gas is used as feedstock. The crude alcohol is imported into Texas and rectified there to a high degree of purity. Because it is of synthetic origin, rather than a fermentation product, it may not be used in beverages, and does not qualify for the tax concessions for use as fuel alcohol, so it is confined to the industrial market. It currently sells at about $1.25 per gallon at 192 proof, or $1.35 at 200 proof, which is about $0.25 below the prevailing prices before the Saudi plant started up. When sold in small quantities or for special purposes, the prices might be as high as $1.50-$1.60, but there is an appreciable element of service involved in the custom denaturing, etc. required.

(c) Fuel alcohol. The fuel alcohol market is dependent on federal and state tax concessions, as ethanol cannot compete directly with gasoline on an equal footing.

There is an exemption of 6 cents out of the 9 cents per gallon federal tax on gasoline if it contains 10% of alcohol. This means that when one gallon of alcohol is blended with nine gallons of gasoline, the blender obtains an exemption equal to 60 cents per gallon of ethanol used. This tax exemption is scheduled to continue until 1992.

There are also state tax exemptions or state grants for fuel alcohol producers in many states, so that the economics of fuel alcohol production will greatly depend on which state it is being produced in, and in which state it is being sold.

Referring to the map of State Tax Incentives for Fuel Alcohol, (Figure 1.), it will be seen that the tax exemptions on 10% alcohol-90% gasoline blends vary from 16 cents per gallon for alcohol produced in Louisiana down to 1 cent per gallon in Iowa. That means that alcohol produced and sold in Louisiana currently obtains a total exemption of $1.60 per gallon when blended with gasoline, which together with the federal tax exemption adds up to $2.20 per gallon. The state tax incentives are effective for varying periods of time, and some states have special phase-out provisions, so it is essential to obtain full details of a state's incentive laws and to seek advice on their operation, as there are various restrictions. In Montana and Utah, for example, there is currently a producer grant of 30 cents per gallon of fuel alcohol produced, and since the laws do not indicate that the alcohol must be sold within the state, it may be sold in a state such as Kansas, where there is a 4 cents per gallon exemption on blends (i.e. 40 cents per gallon of alcohol). It may not, on the other hand, be sold in Idaho, as the incentive there is restricted to alcohol produced within that state.

It will be noted from the map that there is currently no tax incentive for fuel alcohol production or use in the Dairy State of Wisconsin, or in New York, Pennsylvania, or Michigan. In Minnesota, the position is rather complicated, in that there is currently a producer tax credit of 25 cents per gallon of fuel alcohol, which diminishes to 20 cents in 1988, while there will also be a producer grant of 15 cents per gallon in 1987 which increases to 20 cents per gallon in 1988, making a total of 40 cents per gallon.
5. Conclusions

There is no simple answer to the question "Does it pay to make alcohol from whey?" One should conduct a feasibility study for each potential location, which would seek answers to the following questions, amongst others:

(a) What is the current use or method of disposal of whey? What are the costs or returns?
(b) Is there sufficient whey at a single plant to support an alcohol plant?
(c) Would it be necessary to bring in whey from other plants, and at what cost?
(d) Is the whey deproteinized, or otherwise pretreated?
(e) What are the market outlets for the different types of alcohol?
(f) Which type of alcohol would give the best return, after making allowances for the additional capital equipment and operating costs to produce the higher-value grades?

Finally, there is one figure of interest to you that I realize I have not quoted. It is the yield of alcohol which can be expected from whey. With normal, unconcentrated whey, one can make one gallon of anhydrous alcohol from 40 gallons of whey. It may not sound very much expressed like that, but translated into more understandable terms, it means that for every 40 lb. block of cheese coming off your line, there could be sufficient alcohol produced to follow it up with a case of vodka!

Enquiries regarding licensing of the Carbery Whey-Alcohol Process and assistance with initial feasibility studies, etc., should be addressed to the Chief Executive Officer, Carbery Milk Products, Ballineen, Co. Cork, Ireland. (Telephone from U.S.: 011-353-234-7222)
LOW COST WASTEWATER TREATMENT SYSTEM

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Ohio State University
LOW COST WASTEWATER TREATMENT SYSTEM

INTRODUCTION

One of the conditions of the regulations developed by the United States Environmental Protection Agency under the Clean Water Act is development of an approved pretreatment program for industrial dischargers of highly polluted wastewaters. This act also requires municipalities to equitably recover revenue from industrial waste dischargers to pay for public wastewater treatment (CFR, 1979). This results in stiff surcharges on wastewater received from dairy, meat and other food processing facilities that do not pretreat. In some cases, municipalities will not accept the effluent from these processors with or without surcharges unless waste is first pretreated to reduce excessively high concentrations of biochemical oxygen demand (BOD), solids (expressed as SS) and fats, oils and grease (FOG).

Whey and milk or whey permeate are prime examples of materials with very high BOD. If these materials are released to a municipal sewer, surcharges or discharge limits will eventually be incurred. Proper utilization/treatment of these materials is necessary for a cheese plant to remain in business. This paper describes a wastewater pretreatment process which shows potential to reduce or eliminate cheese plant sewer surcharges. There is even potential of making a profit through sale of by-products of whey/permeate treatment. It works for a rendering plant—could it work for whey or permeate?

Often, pretreatment of food processing waste will yield a positive net benefit to the processor. The capital, operation and maintenance costs of pretreatment facilities plus the value of saleable by products of the treatment process are less than sewer surcharge fees (Hansen, et
al., 1984; McVaugh, 1979). Results of a research project on optimization of wastewater treatment for a hog processor, indicated anaerobic pretreatment as the method showing most potential for net gain to the plant (Hansen, et al., 1984).

Anaerobic digestion has been an integral part of wastewater treatment since the early 1900's (McKinney, 1962; LaRiovere, 1978; Speece, 1983). Anaerobic metabolism of organic substrate is characterized by low nutrient requirements, and high energy product formation in the form of methane. Anaerobic metabolism yields 20 to 30 times less energy per unit amount of substrate processed than aerobic metabolism (Dupont, 1983). This low energy yield from anaerobic metabolism results in the conversion of the energy, originally contained in the substrate, to the high energy product methane and new cell material. Food waste treatment facilities that collect and utilize biogas are becoming more numerous (Hills and Roberts, 1981; Ruppel, et al., 1982; and Huss and Ellis, 1985).

The protein containing biomass produced by anaerobic treatment of organic waste is utilizable as animal feed. Possible problems with toxic elements (i.e. Fe, Al, Zn, Cu, Cd) can exist with the consumption of biomass originating from the biological treatment of municipal sewage. However, biomass utilization as an animal feed supplement may have a future with the biological treatment of food processing type wastewaters (Kavanagh et al., 1982; Heddle, 1979). Biomass is created from the microbial conversion of waste materials into quality protein via cell synthesis (Hang, 1979; Litchfield, 1977; Grady and Lim, 1980). Microorganisms in general contain adequate amounts of vitamins, minerals, and essential amino acids to establish biological protein as a suitable animal feed supplement (Waslien, 1975).
The goal of this research was to further develop and demonstrate an effective wastewater treatment system that coproduces saleable biproducts. The system chosen was an upflow anaerobic sludge blanket (UASB) digester. The system was designed with devices to collect biogas for inplant use and biological protein which could be added to meat and bone meal.

EXPERIMENT METHODS

Apparatus

The UASB is an anaerobic treatment process that has important advantages over other types of anaerobic treatment. It is a cell retention process that permits much shorter wastewater retention times than conventional, complete mix digesters. UASB digester vessels are smaller than even fixed-film reactors in which inert media takes up to 75% of the reactor volume (Speece, 1983). Biological protein can be easily harvested from the UASB.

The UASB was developed by Lettinga, et al. (1980). The concept is simple, a dense sludge blanket made up of active biomass develops in the digester vessel through which wastewater flows in an upward direction. A solid/liquid separator allows expulsion of treated effluent while retaining most of the solids which settle back into the sludge blanket.

The initial design of the UASB was according to previous process descriptions (Frostell, 1980; Lettinga, et al., 1980). A 4 l laboratory scale digester with length:diameter ratio of 2 was built. Auxiliary systems included those for feeding and recirculation, temperature control and methane gas collection. Schematics of the digester and gas collection system are given as Figures One and Two. Temperature was maintained at 35°C by a Yellow Springs Instrument Co., Model 71A with
surface probe, Type 408. The recycle line was circulated through an external heat exchanger to facilitate heating. Solid/liquid separation was accomplished with an inverted cone (gas deflector and collector in Fig. 1).

An 1100 l pilot plant was built about six months into the research. The pilot plant was built on-site at a rendering plant and was operated by plant personnel. The pilot plant's design and the concept of operation was similar to the laboratory scale UASB. Design and operation parameters and data for the pilot plant were not released at the time this report was written.

Waste Substrate

Three waste streams from a typical, continuous dry rendering plant were used as substrate. These were cooker condensate, raw blood and "blood wastewater" - liquid material collected from the raw product storage hopper mixed with plant clean-up water in unpredictable proportions. A schematic of a typical continuous dry rendering process is given as Figure 2. Cooker condensate accounts for the major portion of the total wastewater volume with a Chemical Oxygen Demand (COD)) greater than 2500 mg/l. Blood wastewater accounts for most of the remaining high strength wastewater. Raw blood accounts for a small percentage of the volume of high strength wastewater but is very high in COD (140,000 - 180,000 mg/l). Rendering wastewater characteristics are given in Table One. Analyses for COD, FOG, solids (SS, TS and VSS), pH, N (TKN and ammonia - N) and volatile fatty acids (VFA) were performed in our laboratory according to Standard Methods (1975). All other analyses were performed in licensed commercial laboratories.
RESULTS AND DISCUSSION

Data reported here was from four perturbations (experimental runs) in the 4 l digester extending over 10 months. The four runs were distinguished from one another by changes in influent characteristics as shown in Table Two. Preliminary to the first run the digester was brought to steady state using active anaerobic sludge from a sewage treatment plant as starter. For all reported trials, temperature was maintained at 35°C and recycle rate was about 200 ml/min, operated continuously except during loading. The digesters were slug loaded either daily (runs 1-3) or every other day (run 4). Material was added in a manner to preclude washing out cells (West, 1983). Loading parameters and results of experiments are summarized in Tables Two through Four.

The results reported for runs one through three (Table Three) were taken after the system had reached steady state. These runs used similar components in the feed (influent); blood wastewater and cooker condensate, though the percentages of these components were varied. Raw blood was added to the feed in run four and the digester would not come to steady state. This was predictable based on related experiments in the 1100 l pilot plant (data not reported) which was operating by that time. The influent COD in the laboratory scale digester for run four was increased slightly during the run so as to be more similar to conditions for the pilot plant.

It would appear from data for run four in the 4 l digester (Table Four) and from observations made in the pilot plant that the UASB digester could not assimilate 5% raw blood for extended periods of time. COD level in the digester influent increased from 11,100 to 14,000 mg/l
(Figure 2) and efficiency of COD removal decreased significantly as indicated by the increase in effluent COD (Figure 2 and Table 4). Biogas production also decreased (Table Four), further indication that the digester was under severe stress. Data from runs one and two would indicate that the UASB digester could handle at least twice the COD loading of rendering waste than was used in run four, therefore excessive COD was not the major factor in the digester failure though it may have contributed. Excessive ammonia liberation with a concurrent increase in pH was the most likely cause of the drop in digester performance.

A large part of the COD of rendering waste is due to the presence of proteins. Raw blood is high in protein, about 70% crude protein (organic N * 6.25) on a dry basis, which decomposes releasing ammonia from the deamination of amino acids. The feed of run four contained 1460 mg-N/l of total nitrogen, which explains the buildup of ammonia within the reactor. Ammonia levels had climbed to 1170 mg-N/l at the end of run four with the pH exceeding 8.0 (Figure two). This would correspond to free ammonia concentrations on the order of 60 mg-N/l. Adjusting pH of the digester with dilute acetic acid and a reduction in the loading rate restored reactor performance almost immediately.

Mean values of biogas production per mass of COD loaded into the digester and per mass of COD converted (\(\text{COD}_{\text{total (influent)}} - \text{COD}_{\text{total (effluent)}} = \text{COD}_{\text{converted}}\)) is given in Table Five. Gas production for run four before the digester began to fail was similar to those in Table Five. This range of values for gas production per mass of COD converted is within the range reported for 39 large commercial UASB digesters treating primarily carbohydrate wastes (Sax, 1985).
Samples of the sludge bed within the UASB reactor were withdrawn prior to run four to examine the yield and quality of biological protein available. Approximately 26 g of biological biomass containing 24% protein (30% crude protein as TKN * 6.25) was available from the digester per kg of COD converted. The amino acid analysis for this material is shown in Table Six. Table Seven contains the amino acid profiles for some common animal feed and protein supplements for comparison purposes to biological protein. Table Eight shows the mean of essential amino acid profiles for aerobic sludge from two activated sludge plants and one trickling-filter plant treating meat packing wastewater (Kavanagh, et al., 1982). Based upon comparison of amino acid profiles the UASB biological protein (BP) is obviously very high quality. The BP contains relatively high concentrations of isoleucine, leucine, lysine, methionone, threonine and tryptophan, amino acids that must often be added as supplements to animal feeds at additional expense.

The essential amino acid profile of BP resembles very closely the profiles associated with the aerobic sludge from the meat packing facilities. It may be possible that part of the BP is actually a humus type material derived from blood. However, the similarities between the amino acid profiles of blood meal and the biological sludges are inconclusive.

Limited feeding trials of biological protein have shown no real clinical difference in animal weight gain or overall health (Kavanagh et al., 1979). In a preliminary experiment in our laboratory, biological protein from the UASB was free choice fed to young male and female mice at about 15% of ration, the balance being commercial mouse feed. Results of a two month trial indicated no clinical difference between a
control population and the group fed BP supplement. These results, give encouragement that BP might be a viable feed supplement, however there exists questions of safety in regards to animal health and total digestibility of the material. It also remains to be determined if key vitamins and minerals are present and the amount of non amino acid protein contained in the biological protein.

SUMMARY

The UASB digester in an 8 day hydraulic retention time is stable in removing 90% of the COD from rendering wastewater with an influent COD over 10,000 mg/l. The process failed with an influent containing cooker condensate and 5% raw blood (COD greater than 11,000 mg/l, total nitrogen over 1100 mg-N/l) at a 16 day hydraulic retention time. The apparent cause of failure was a buildup of ammonia in the digester and a concurrent increase in pH to 8.1. An approach for future research would be to optimize the COD:N:P ratio by proper mixing of the rendering wastewater components to achieve a stable system.

The UASB digester produced 0.34-0.39 l of biogas (about 75% methane) per gram of COD converted and 0.28-0.31 l of biogas per gram of COD influent. The biological protein (biomass) contained 24% protein that was notably high in isoleucine, leucine, lysine, methionine, tryptophan, and threonine. Additions of biological protein to meat/bone meal would enhance the amino acid profile of the meal.

RECOMMENDATIONS FOR ADDITIONAL RESEARCH WITH WHEY AND PERMEATE

The UASB has been shown to be effective in treating general dairy processing waste which is part whey. There needs to be a comprehensive research study to test the UASB in testing full strength whey or permeate. For industrial pretreatment purposes it is best to separate
out the strongest (most polluted) waste streams for treatment in on-site, relatively small, inexpensive digesters.

There is a need to examine the suitability of using whey and/or permeate as the sole carbon and nutrient source for manufacture of biological protein and biogas. Harvesting biological protein and biogas can substantially improve the net gain to industry compared to the anaerobic digestion processes presently used which only collect biogas.

The biological protein produced by anaerobic digestion of whey or permeate should be studied to determine suitability as an animal feed. These studies should include amino acid and non protein nitrogen analysis and a determination of digestibility of the material. Health and safety concerns must also be considered.

Examination of the process of digesting whey and permeate in a UASB system may help the cheese industry almost immediately. However, the greater benefit may come from better understanding of the factors (i.e. limiting nutrients or micro nutrients, precise control of intensive properties, flow dynamics of the fermenter, etc.) that now limit the use of whey or permeate as a general carbon and nutrient source for industrial fermentations. This may lead to utilization of whey as a substrate for many types of biotechnological applications such as manufacture of chemicals or biological treatment of hazardous wastes.
REFERENCES


2. CFR. 1985. 40, 403.


Figure 1 : Schematic of operating system.
Figure 2. Flow diagram of a rendering plant with three separate high-strength wastewater streams.
### Table One. Rendering Wastewater Characteristics

<table>
<thead>
<tr>
<th>Component</th>
<th>Cooker Condensate</th>
<th>Blood Wastewater</th>
<th>Raw Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>(All units are mg/l except pH)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical Oxygen Demand</td>
<td>3,000 (2600-9800)</td>
<td>100,000 (20,000-100,000)</td>
<td>140,000-180,000</td>
</tr>
<tr>
<td>Fats, Oils, Grease</td>
<td>300</td>
<td>3,500 (2200-8200)</td>
<td>N/A</td>
</tr>
<tr>
<td>pH</td>
<td>8.5 (8.0-9.0)</td>
<td>6.0 (5.5-6.5)</td>
<td>6.7 (6.5-6.9)</td>
</tr>
<tr>
<td>Total Solids</td>
<td>N/A*</td>
<td>21500 (4300-46500)</td>
<td>145,000</td>
</tr>
<tr>
<td>Volatile Solids</td>
<td>N/A*</td>
<td>18600 (4100-38400)</td>
<td>N/A</td>
</tr>
<tr>
<td>TKN</td>
<td>600</td>
<td>3,350</td>
<td>16,800</td>
</tr>
<tr>
<td>Organic N</td>
<td>0</td>
<td>2,980</td>
<td>16,500</td>
</tr>
<tr>
<td>Ammonium N</td>
<td>600</td>
<td>370</td>
<td>300</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0</td>
<td>2,560</td>
<td>260</td>
</tr>
<tr>
<td>Potassium</td>
<td>0</td>
<td>470</td>
<td>1,160</td>
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<td>Calcium</td>
<td>1</td>
<td>150</td>
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</tr>
<tr>
<td>Magnesium</td>
<td>0</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>Chloride</td>
<td>-</td>
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</tr>
<tr>
<td>Zinc</td>
<td>0</td>
<td>1.7</td>
<td>0.03</td>
</tr>
<tr>
<td>Copper</td>
<td>0</td>
<td>0.4</td>
<td>1,300</td>
</tr>
<tr>
<td>Lead</td>
<td>0</td>
<td>0.2</td>
<td>N/A</td>
</tr>
<tr>
<td>Chromium</td>
<td>0</td>
<td>0.2</td>
<td>0.14</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.0</td>
<td>0.005</td>
<td>0.044</td>
</tr>
<tr>
<td>Nickel</td>
<td>0</td>
<td>0.05</td>
<td>0.29</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.1</td>
<td>880</td>
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</tr>
<tr>
<td>Magnanese</td>
<td>0.003</td>
<td>0.9</td>
<td>N/A</td>
</tr>
<tr>
<td>Iron</td>
<td>0.42</td>
<td>100</td>
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<tr>
<td>Boron</td>
<td>0.01</td>
<td>0.3</td>
<td>N/A</td>
</tr>
<tr>
<td>Cobalt</td>
<td>0.01</td>
<td>0.02</td>
<td>N/A</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>0.02</td>
<td>0.07</td>
<td>N/A</td>
</tr>
<tr>
<td>Sulfate Sulfur</td>
<td>2</td>
<td>-</td>
<td>N/A</td>
</tr>
<tr>
<td>Total Sulfur</td>
<td>-</td>
<td>250</td>
<td>N/A</td>
</tr>
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</table>

*Standard total solids test gives a value of approx. 0, but this is due to the loss of volatiles (mainly fat and oils) during testing.
Table Two. Influent Parameters for Experimental Runs in a Laboratory Scale UASB Digester

<table>
<thead>
<tr>
<th>Experimental Runs</th>
<th>1</th>
<th>2</th>
<th>3&lt;sup&gt;a&lt;/sup&gt;</th>
<th>4&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD Loading Rate (kg/m&lt;sup&gt;3&lt;/sup&gt;-day)</td>
<td>1.4</td>
<td>1.1</td>
<td>0.47</td>
<td>0.88/0.88&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Duration of run (d)</td>
<td>47</td>
<td>27</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td>Influent (ml/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Wastewater</td>
<td>200</td>
<td>35</td>
<td>20/35</td>
<td>-</td>
</tr>
<tr>
<td>COD (mg/l)</td>
<td>23,500</td>
<td>99,800</td>
<td>40,000</td>
<td>-</td>
</tr>
<tr>
<td>FOG (mg/l)</td>
<td>2,200</td>
<td>6,400</td>
<td>N/A</td>
<td>-</td>
</tr>
<tr>
<td>TKN (mg/l)</td>
<td>1,500</td>
<td>3,000</td>
<td>2,000</td>
<td>-</td>
</tr>
<tr>
<td>Cooker Condensate</td>
<td>300</td>
<td>200</td>
<td>100/200</td>
<td>113/238&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>COD (mg/l)</td>
<td>3,200</td>
<td>5,300</td>
<td>5,300</td>
<td>225/475</td>
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<tr>
<td>FOG (mg/l)</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>TKN (mg/l)</td>
<td>600</td>
<td>650</td>
<td>650</td>
<td>650</td>
</tr>
<tr>
<td>Raw Blood</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25</td>
</tr>
<tr>
<td>COD (mg/l)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>170,000</td>
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<tr>
<td>Distilled Water</td>
<td>265</td>
<td>130/265</td>
<td>250/0</td>
<td>250/0</td>
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<tr>
<td>Composited Influent</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Volume (ml/d)</td>
<td>500</td>
<td>500</td>
<td>250/500</td>
<td>500</td>
</tr>
<tr>
<td>How Loaded</td>
<td>Daily</td>
<td>Daily</td>
<td>Daily</td>
<td>Alternate Days</td>
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<tr>
<td>Hydradulic Retention time (d)</td>
<td>8</td>
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<tr>
<td>COD (mg/l)</td>
<td>11,500</td>
<td>9,100</td>
<td>5,050</td>
<td>11,100&lt;sup&gt;a,b&lt;/sup&gt;</td>
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<tr>
<td>FOG (mg/l)</td>
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<td>600</td>
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<td>N/A</td>
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<tr>
<td>TKN (mg/l)</td>
<td>960</td>
<td>470</td>
<td>410</td>
<td>1130/1460</td>
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<tr>
<td>COD:TKN</td>
<td>12</td>
<td>19.4</td>
<td>12.4</td>
<td>9.8/9.6</td>
</tr>
</tbody>
</table>

<sup>a</sup>Digester was fed alternate daily slug loads of 250 ml and 500 ml.
<sup>b</sup>Influent COD was increased about 25% early in the run by replacing distilled water with cooker condensate in the influent.
### Table Three. Effluent Characteristics for Runs One - Three

<table>
<thead>
<tr>
<th>Effluent Characteristics</th>
<th>Experimental Runs</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>COD - Total (mg/l)</td>
<td>800 (600-1000)</td>
</tr>
<tr>
<td>COD - Soluble (mg/l)</td>
<td>550 (400-700)</td>
</tr>
<tr>
<td>TS (mg/l)</td>
<td>N/A</td>
</tr>
<tr>
<td>VS (mg/l)</td>
<td>N/A</td>
</tr>
<tr>
<td>TSS (mg/l)</td>
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</tr>
<tr>
<td>VSS (mg/l)</td>
<td>N/A</td>
</tr>
<tr>
<td>NH₃ (mgN/l)</td>
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</tr>
<tr>
<td>TKN (mg N/l)</td>
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</tr>
<tr>
<td>Alkalinity (mg CaCO₃/l)</td>
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</tr>
<tr>
<td>pH</td>
<td>7.2-7.5</td>
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Biogas Production (ml/d at standard temp & press)

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Maximum</th>
<th>Average</th>
<th>Methane (%)</th>
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</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>1350</td>
<td>1000</td>
<td>410</td>
<td></td>
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<tr>
<td>Maximum</td>
<td>2520</td>
<td>1430</td>
<td>600</td>
<td></td>
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<tr>
<td>Average</td>
<td>1800</td>
<td>1260</td>
<td>520</td>
<td></td>
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<tr>
<td>Methane (%)</td>
<td>70-75</td>
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<td>77</td>
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COD reduction (%)

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<tr>
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<th>Total</th>
<th>Soluble</th>
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<tbody>
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<td>94-97</td>
<td>78-82</td>
</tr>
<tr>
<td></td>
<td>86-92</td>
<td></td>
</tr>
<tr>
<td>Day Number</td>
<td>Feed COD Concentration (mg/l)</td>
<td>Effluent COD (total) (mg/l)</td>
</tr>
<tr>
<td>------------</td>
<td>-------------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>0</td>
<td>11,100</td>
<td>510</td>
</tr>
<tr>
<td>2</td>
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<td>16</td>
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<td>1700</td>
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<tr>
<td>18</td>
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<td>1600</td>
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<td>20</td>
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<td>2150</td>
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<tr>
<td>22</td>
<td>&quot;</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>&quot;</td>
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Table Five. Mean Values of Biogas\(^a\) Production per Mass of COD Loaded and Converted

<table>
<thead>
<tr>
<th>Trial</th>
<th>COD loaded ((1/g , \text{COD}_i)^b)</th>
<th>COD converted ((1/g , \text{COD}_c)^c)</th>
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<tbody>
<tr>
<td>1</td>
<td>0.31</td>
<td>0.34</td>
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<td>2</td>
<td>0.28</td>
<td>0.39</td>
</tr>
<tr>
<td>3</td>
<td>0.28</td>
<td>0.34</td>
</tr>
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</table>

\(^a\)About 75\% methane  
\(^b\)\text{COD}_i = \text{COD}, \text{loaded}  
\(^c\)\text{COD}_c = \text{COD}, \text{converted}
Table Six. Amino Acid Profile for Biological Protein

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>% w/w*</th>
<th>g/100 g protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>1.67</td>
<td>7.00</td>
</tr>
<tr>
<td>Valine</td>
<td>1.58</td>
<td>6.62</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.40</td>
<td>5.87</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.19</td>
<td>4.99</td>
</tr>
<tr>
<td>Leucine</td>
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<td>8.71</td>
</tr>
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<td>Proline</td>
<td>0.98</td>
<td>4.11</td>
</tr>
<tr>
<td>Threonine</td>
<td>1.22</td>
<td>5.11</td>
</tr>
<tr>
<td>Serine</td>
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<td>4.36</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.51</td>
<td>2.14</td>
</tr>
<tr>
<td>Hydroxyproline</td>
<td>0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>Phenylalanine</td>
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<td>5.23</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>2.66</td>
<td>11.14</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>2.66</td>
<td>11.14</td>
</tr>
<tr>
<td>Tyrosine</td>
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</tr>
<tr>
<td>Lysine</td>
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<td>Histidine</td>
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</tr>
<tr>
<td>Arginine</td>
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<td>4.65</td>
</tr>
<tr>
<td>Cystine/2</td>
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<td>1.30</td>
</tr>
<tr>
<td>Tryptophan</td>
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</table>

* Amino acid weight percentage of total weight of dried material
Table Seven. Amino Acid Profile (g/100 g protein) for Animal Feed and Feed Supplements

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Blood Meal</th>
<th>Meat and Bone Meal</th>
<th>Milk Dried</th>
<th>Corn Grin</th>
<th>Wheat Grin</th>
<th>Whey Dried</th>
<th>Soybean Meal, Solv.</th>
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</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>-</td>
<td>7.80</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Arginine</td>
<td>4.38</td>
<td>6.16</td>
<td>3.58</td>
<td>5.68</td>
<td>5.59</td>
<td>2.90</td>
<td>6.99</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>-</td>
<td>7.76</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cystine</td>
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<td>0.92</td>
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<td>2.17</td>
<td>1.46</td>
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<tr>
<td>Glutamic Acid</td>
<td>-</td>
<td>12.22</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Glycine</td>
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<td>14.82</td>
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<td>7.01</td>
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<td>6.20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Isoleucine</td>
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<tr>
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<td>5.91</td>
<td>9.85</td>
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<td>10.14</td>
<td>7.42</td>
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<td>Lysine</td>
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<td>5.56</td>
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<td>2.27</td>
<td>3.54</td>
<td>7.97</td>
<td>6.33</td>
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<tr>
<td>Methionine</td>
<td>1.13</td>
<td>1.41</td>
<td>2.39</td>
<td>1.93</td>
<td>1.42</td>
<td>1.45</td>
<td>1.31</td>
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<tr>
<td>Phenylalanine</td>
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<td>3.43</td>
<td>4.48</td>
<td>5.68</td>
<td>4.88</td>
<td>2.90</td>
<td>4.80</td>
</tr>
<tr>
<td>Proline</td>
<td>-</td>
<td>8.95</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Serine</td>
<td>-</td>
<td>3.95</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Threonine</td>
<td>4.63</td>
<td>3.16</td>
<td>4.18</td>
<td>4.54</td>
<td>2.83</td>
<td>5.80</td>
<td>3.71</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1.38</td>
<td>0.90</td>
<td>1.19</td>
<td>1.14</td>
<td>1.42</td>
<td>1.45</td>
<td>1.31</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.25</td>
<td>2.16</td>
<td>3.88</td>
<td>-</td>
<td>3.54</td>
<td>2.17</td>
<td>3.06</td>
</tr>
<tr>
<td>Valine</td>
<td>8.14</td>
<td>4.10</td>
<td>6.57</td>
<td>4.54</td>
<td>4.17</td>
<td>5.07</td>
<td>5.24</td>
</tr>
<tr>
<td>Crude Protein %</td>
<td>79.9</td>
<td>51.95(^a)</td>
<td>33.5</td>
<td>8.8</td>
<td>12.7</td>
<td>13.8</td>
<td>45.8</td>
</tr>
</tbody>
</table>

Data from Church (1984).
Data from a local rendering company.
True amino acid content.
Table Eight. Essential Amino Acid Profile (g/100 g protein) for Aerobic Sludge from Several Meat Packing Wastewater Treatment Facilities

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Activated Sludge $^1$</th>
<th>Trickling Filter Sludge</th>
<th>Activated Sludge $^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>7.3</td>
<td>7.4</td>
<td>4.6-6.1</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.4</td>
<td>1.1</td>
<td>1.3-1.9</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
</tr>
<tr>
<td>Arginine</td>
<td>3.2</td>
<td>5.0</td>
<td>3.2-4.6</td>
</tr>
<tr>
<td>Threonine</td>
<td>5.4</td>
<td>5.3</td>
<td>3.5-5.0</td>
</tr>
<tr>
<td>Cystine</td>
<td>1.0</td>
<td>0.9</td>
<td>0.0</td>
</tr>
<tr>
<td>Valine</td>
<td>5.3</td>
<td>5.3</td>
<td>3.3-8.6</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.0</td>
<td>1.7</td>
<td>tr-1.8</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3.3</td>
<td>3.2</td>
<td>3.7-.41</td>
</tr>
<tr>
<td>Leucine</td>
<td>6.6</td>
<td>6.3</td>
<td>5.6-6.5</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>4.0</td>
<td>3.9</td>
<td>2.5-3.1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>4.5</td>
<td>3.9</td>
<td>3.7-3.8</td>
</tr>
<tr>
<td>Crude Protein (%)</td>
<td>39.4</td>
<td>23.8</td>
<td>45-60</td>
</tr>
</tbody>
</table>

$^1$Kavanagh et al. (1982)
$^2$Heddle (1979)
The 8th edition of Bergey's Manual of Determinative Bacteriology shows the genus *Listeria* with four species: *L. monocytogenes*, *L. denitrificans*, *L. Grayi*, and *L. murrayi*. Although the inclusion of *L. denitrificans* in the genus *Listeria* is now generally questioned, it continues to bear that name until reclassified (1). Stuart and Welshimer (2) have proposed a new family, *Listeriaceae*, to contain two monospecific genera, *Listeria* and *Murraya*. Under this classification system, *L. monocytogenes* would contain a heterogeneous group of organisms distinguishable from *Murraya* by their inability to ferment mannitol. *Murraya* would contain two subspecies, *M. grayi ssp. grayi* and *M. grayi ssp. murrayi*, with the two distinguishable by the ability of *M. grayi ssp. murrayi* to reduce nitrates. In this chapter we list the above two subspecies by the older notation of *L. murrayi* and *L. grayi*, solely for compatibility with the older literature.

Two groups within *L. monocytogenes* as proposed by Stuart and Welshimer are readily apparent, the hemolytic pathogenic strains and the nonhemolytic apathogenic strains. The literature contains frequent references to "nonpathogenic" *L. monocytogenes*. This group became known as *L. innocua* from the Latin word for harmless. The type strain of the species was described by Seeliger (3). Two other apathogenic species are now recognized, *L. welshimeri* and *L. seeligeri* (4).

The most recently described species is *L. ivanovii* (5), a strongly hemolytic genomic group formerly called *L. monocytogenes* serovar 5 and *L. bulgarica*. It is associated with animal pathology and rarely isolated from human sources. Only *L. monocytogenes* is consistently associated with human illness. Both
L. ivanovii and L. monocytogenes are pathogenic for mice. L. denitrificans is also a mouse pathogen, but generally accepted as nonpathogenic for man. The accepted Listeria species and related organisms are shown in Table 1.

The classical tests for Listeria pathogenicity are the Anton test (rabbits), inoculation of mice and inoculation of embryonated eggs (6). The mouse test (i.p. injection) is most frequently used, and is described in this chapter.

Animal confirmation of L. monocytogenes pathogenicity is not routinely done for clinical isolates, and it is optional for regulatory sample isolates. Virulence confirmation is not required, and one should not hesitate to call an isolate L. monocytogenes if it meets all the other criteria outlined in this chapter.

Table 2 contains the information relating to serological characterization of Listeria species. Most of the L. monocytogenes isolates from patients and the environment are type 1 or 4. Better than 90% of L. monocytogenes isolates can be serotyped using commercial (Difco) sera. It should be noted that all the nonpathogenic species, except L. welshimeri, share one or more somatic antigens with L. monocytogenes. Serotyping alone without thorough characterization is not adequate for identification of L. monocytogenes.

Of the biochemical test, the rhamnose and xylose fermentation pattern is essential for differentiating the Listeria species. An exception is L. monocytogenes and L. innocua which both fail to ferment xylose. These two species can be separated, however, by the absence of hemolytic activity in L. innocua, as demonstrated by sheep blood stab or the CAMP test. L. seeligeri, on the other hand, may be slightly hemolytic on sheep blood agar. Since it shares antigens with L. monocytogenes, it might be incorrectly
identified without the xylose-rhamnose reactions and/or mouse pathogenicity. It is impossible to over emphasize the importance of doing a complete characterization of each isolate. Partial characterization, although accurately done, may be misleading.

The CAMP test (Table 3) is useful in confirming species. The test is performed by streaking on a sheep blood agar plate a β-hemolytic *Staphylococcus aureus* and a *Rhodococcus equi* culture vertically and test cultures horizontally between them. After incubation at 35°C for 24-48 h, the plates are examined for hemolysis surrounding the test cultures in the vicinity of the zone of influence of the vertical streaks. *L. monocytogenes* hemolysis is enhanced in the vicinity of the *Staphylococcus* streak. *L. ivanovii* hemolysis is enhanced in the vicinity of the *Rhodococcus* streak, *L. seeligeri* becomes hemolytic in the vicinity of the *Staphylococcus* streak. The other species remain nonhemolytic.

There is considerable interest in simplifying and shortening the isolation procedure for *L. monocytogenes*, and a few people are working on answers to the problem. The most promising is a DNA probe under development by Dr. Michael Thomashow, Washington State University. He has cloned the hemolysin gene from a serotype 1 *L. monocytogenes*. The probe is now too large and nonspecific for practical use. Subcloning for specificity looks promising.

When handling, storing, and shipping materials to be analyzed for Listeria, refrigeration at 4°C is recommended. *Listeria* will grow, although slowly, at this temperature. Long periods of holding in the cold is frequently recommended as the best method of enriching the organism in materials likely to contain large numbers of competitors.
A. Equipment and materials

1. Balance (gram range)
2. Cover slips, glass
3. Erlenmeyer flask, 500 ml
4. Fermentation tubes (Durham)
5. Grease pencil or magic marker
6. Incubator, 30°C
7. Incubator, 35°C
8. Immersion oil
9. Inoculating loops (NUNC), plastic, disposable
10. Inoculating needle
11. Microscope slides
12. Needle, 26 gauge, 3/8 inch
13. Phase microscope with oil immersion objective
14. Petri plates
15. Pipet, 25 ml
16. Pipet, 10 ml
17. Pipet, 1 ml
18. Screw-cap tubes, 16 x 125 mm
19. Stomacher
20. Stomacher bag
21. Syringe, tuberculin, sterile, disposable

B. Media and reagents

1. Acetic acid, 5 N
2. Acriflavin HCl (Sigma)
3. Agar (Difco Bacto)
4. α-naphthylamine
5. α-naphthol
6. Blood agar base #2 (Oxoid)
7. Cycloheximide (Sigma)
8. Defibrinated sheep blood
9. Dextrose
10. Esculin
11. Ethanol
12. FA buffer (Difco)
13. Glycine anhydride (Sigma)
14. Gram stain kit
15. Hydorgen peroxide
16. KOH
17. Listeria-typing sera set (Difco)
18. Lithium chloride (Sigma)
19. Maltose
20. Mannitol
21. Methyl red
22. MR-VP broth
23. Naladixic acid (Sodium salt, Sigma)
24. NaCl
25. Nutrient broth (Difco)
26. Phenylethanol agar (Difco)
27. Purple broth base (BBL)
28. Rhamnose
29. SIM (BBL)
30. Sulfanilic acid
31. Trypticase soy broth (BBL) (M137)
32. Trypticase soy agar (BBL) (M136)
33. Tryptose (Difco Bacto)
34. Triple sugar iron agar (BBL or Difco)
35. Xylose
36. Yeast extract (BBL)

C. Enrichment procedure
Add 25-ml liquid or 25-g cream or solid to 225-ml enrichment broth (EB) in blender or stomacher. Blend or stomach as required for thorough mixing. EB culture may be incubated in blender jar or stomacher bag, or transferred to a 500-ml Erlenmeyer flask. Incubate EB culture for 7 days at 30°C.

D. Isolation procedure
1. At 24 h and 7 days, streak EB culture onto modified McBride agar (MMA) both undiluted and diluted 1:10 in 0.5% KOH. Do not delay between diluting and streaking. Incubate MMA plates at 35°C for 48 h.

2. Examine MMA plates for suspect colonies using beamed white light powerful enough to illuminate plate well, striking plate bottom at a 45° angle (Figure 1). When examined in this oblique-transmitted light from an eye position directly above the plate, Listeria colonies appear blue-gray to blue. Pick 5 typical colonies to Trypticase soy agar (M136) with 0.6% yeast extract (TSA-YE), streaking for separation. Incubate TSA-YE plates at 30°C for 24 h or until growth is satisfactory.
E. Identification procedure

1. Examine the TSA-YE plates for typical colonies using the light arrangement already described.

2. Pick a typical colony and do a wet mount examination using 0.85% saline for the suspending medium and for the oil immersion objective of a phase-contrast microscope. *Listeria* appears as slim, short rods with slight rotating or tumbling motility. Always compare to a known culture. Cocci, large rods, or rods with rapid, swimming motility are not *Listeria*.

3. Test a typical colony for catalase. *Listeria* is catalase positive.

4. Do a gram stain. *Listeria* is a small, gram-positive rod.

5. Inoculate a tube of Trypticase soy broth (M137) supplemented with 0.6% yeast extract (TSB-YE) to be used for inoculating biochemicals and fermentations. Incubate at 35°C for 24 h. If needed, this culture may be kept at 4°C for several days and used as inoculum.

6. Inoculate sheep blood agar by stabbing plates poured thick and dried well (check for moisture before using). Draw a grid of 20-25 spaces on the plate bottom. Stab one culture per grid. Always stab positive controls (*L. ivanovii* and *L. monocytogenes*) and a negative control (*L. innocua*). Incubate for 48 h at 35°C.

7. Examine blood agar plates containing culture stabs using a bright light. *L. monocytogenes* produces a slight cleared zone around the stab. *L. innocua* shows no zone of hemolysis, whereas *L. ivanovii* produces a well defined zone of clearing around the stab. Do not try to differentiate species at this point but note nature of the hemolytic reaction.
8. Using the TSB-YE culture, inoculate urea agar slant by covering the slant well without stabbing the butt. Incubate at 35°C for 5 days. Observe daily for development of a purple color (a positive test). *Listeria* does not hydrolyze urea and no color should develop.

9. Using TSB-YE culture, inoculate nitrate broth (M96). Incubate at 35°C for 5 days. Add 0.2 ml reagent A, followed by 0.2 ml reagent B. A red color indicates the presence of nitrite, i.e., nitrate has been reduced. If no color develops, add powdered zinc and let stand 1 h. A developing red color indicates that nitrate is still present and has not been reduced. Only *L. denitrificans* and *L. murrayi* reduce nitrates.

10. Inoculate SIM motility medium from TSB-YE. Incubate for 7 days at room temperature. Observe daily. *Listeria* is motile, giving typical umbrella growth pattern.

11. Inoculate an MR-VP medium (M92) tube using the TSB-YE culture. Incubate at 35°C for 48 h. Remove 1 ml to a clean tube and add 0.6 ml α-naphthol solution followed by 0.2 ml 40% KOH solution. Shake and examine for a strong red color, a positive test. Incubate the remaining culture for an additional 2 days. Add methyl red to tube; it should turn red for a positive test. Except for *L. denitrificans*, *Listeria* is both MR and VP positive.

12. Using the TSB-YE culture, inoculate TSI (M134) by streaking the slant and stabbing the butt. Incubate up to 5 days at 35°C. *Listeria* gives an acid slant and an acid butt, no H₂S.

13. From TSB-YE culture, inoculate the following carbohydrates set up as 0.5% solutions in purple carbohydrate broth (M116): dextrose, esculin, maltose, rhamnose, mannitol, and xylose. Incubate 7 days at 35°C. *Listeria* produces acid with no gas or no reaction. Consult Table 1 for the xylose-rhamnose reactions of the *Listeria* species. All
species should be positive for dextrose, esculin, and maltose. All Listeria species except L. grayi and L. murrayi should be mannitol negative.

F. Serology.

Use TSB-YE culture to inoculate tryptose broth. Make 2 successive transfers of 24 h at 35°C in tryptose broth. Make a final transfer to 2 tryptose agar slants and incubate 24 h at 35°C. Wash both slants in a total of 3 ml Difco FA buffer and transfer to a sterile 16 x 125 mm screw-cap tube. Heat in water bath at 80°C for 1 h. Spin at 1600 x g for 30 min. Remove 2.2-2.3 ml supernate and resuspend pellet in the remainder of the buffer. Follow manufacturer's recommendations for sera dilution and agglutination procedure.

G. Mouse pathogenicity (optional)

Grow isolate for 24 h at 35°C in TSB-YE. Transfer to 2 tubes of TSB-YE for another 24 h at 35°C. Put a total of 10 ml culture broth from both tubes into a 16 x 125 mm tube and spin at 1600 x g for 30 min. Discard supernate and resuspend pellet in 1 ml 0.85% saline diluent. This suspension will contain approximately $10^{10}$ bacterial/ml. Inject (i.p.) 16-18 g Swiss White mice, 5 mice/culture, with 0.1 ml of the concentrated suspension. Each mouse will receive $10^9$ bacterial cells. Observe for death over a 7-day period. Nonpathogenic strains will not kill, but $10^9$ pathogenic cells will kill, usually within 5 days. This test should be controlled with known pathogenic and apathogenic strains.

H. CAMP test*

Streak a β-hemolytic S. aureus (CIP 5710 or NCTC 7 428) and a R. equi (NCTC 1621) vertically on sheep blood. Separate vertical streaks so that test strains may be streaked horizontally between them but not quite touching the

* CAMP test cultures of Staphylococcus and Rhodococcus are available from the Division of Microbiology, FDA, 200 C Street, SW, Washington, DC 20204.
vertical streaks. After 24-48 h incubation at 35°C, examine plates for hemolysis in the zone of influence of the vertical streaks. \textit{L. monocytogenes} hemolysis is enhanced in the vicinity of the \textit{Staphylococcus} streak; \textit{L. ivanovii} hemolysis is enhanced in the vicinity of the \textit{Rhodococcus} streak; and \textit{L. seeligeri} has enhanced hemolysis near the \textit{Staphylococcus} streak. The other species remain nonhemolytic in this test. The CAMP test can differentiate between \textit{L. ivanovii} and \textit{L. seeligeri} and can differentiate between a weakly hemolytic \textit{L. seeligeri} that may have been read as nonhemolytic and \textit{L. welshimeri}. Isolates giving reactions typical for \textit{L. monocytogenes} except for the hemolysin production should be CAMP tested before they are deemed to be nonhemolytic \textit{L. innocua}.

I. Interpretation of analyses data for speciation

All \textit{Listeria} sp. are small, gram-positive rods that demonstrate motility in wet mount and in SIM. They are catalase positive, do not hydrolyze urea, and produce acid slant and acid butt in TSI without production of H$_2$S. They utilize dextrose, esculin, and maltose, and some species utilize mannitol, rhamnose, and xylose with production of acid. All species except \textit{L. denitrificans} (which shows +/-) give +/+ reactions in MR-VP broth. An isolate utilizing mannitol with acid production is \textit{L. grayi} or \textit{L. murrayi}. Nitrate reduction can differentiate between the two since \textit{L. murrayi} reduces nitrate. An isolate-reducing nitrate is either \textit{L. murrayi} or \textit{L. denitrificans}. Mannitol utilization can differentiate between the two since \textit{L. denitrificans} cannot utilize mannitol. In addition, the MR-VP reaction of \textit{L. denitrificans} is different.

\textit{L. monocytogenes}, \textit{L. ivanovii}, and \textit{L. seeligeri} (weak) produce hemolysis in sheep blood stabs and are appropriately CAMP test positive. Of the three,
only L. monocytogenes fails to utilize xylose and is positive for rhamnose utilization. The difficulty in differentiating L. ivanovii from L. seeligeri can be resolved in the CAMP test. L. seeligeri shows enhanced hemolysis at the Staphylococcus streak. L. ivanovii shows enhanced hemolysis at the Rhodococcus streak.

Of the nonhemolytic species, L. innocua may give the same rhamnose-xylose reactions as L. monocytogenes but is negative in the CAMP test. L. innocua is the only species sometimes giving negative results for utilization of both rhamnose and xylose. L. welshimeri that is rhamnose negative may be confused with a weakly hemolytic L. seeligeri unless the CAMP test is run.

After all other results are available, the serotyping of isolates becomes meaningful. All biochemical, serological, and pathogenicity data are summarized in the attached three tables. Complete all data collection before making species determinations.
### Table: Differentiating *Listeria* species and Related Organisms Still Referred to as *Listeria* 

<table>
<thead>
<tr>
<th>Species Designation</th>
<th>Hemolytic Reduction</th>
<th>Nitrate Reduction</th>
<th>Mannitol Utilization</th>
<th>MR/VP Utilization</th>
<th>Rhamnose Utilization</th>
<th>Xylose Utilization</th>
<th>Mouse Pathogen</th>
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<tbody>
<tr>
<td>L. monocytogenes</td>
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<td>+/-</td>
<td>+</td>
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</tr>
<tr>
<td>L. ivanovii</td>
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<td>-</td>
<td>-</td>
<td>+/-</td>
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</tr>
<tr>
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<td>-</td>
<td>+/-</td>
<td>V</td>
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</tr>
<tr>
<td>L. welshimeri</td>
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<td>-</td>
<td>-</td>
<td>+/-</td>
<td>V</td>
<td>+</td>
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</tr>
<tr>
<td>L. seeligeri</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<td>L. grayi</td>
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<td>L. murrayi</td>
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</tbody>
</table>

* - Although pathogenic for mice, it is not believed to be a human pathogen.
<table>
<thead>
<tr>
<th>Listeria Species</th>
<th>Serotype</th>
<th>Sheep Blood</th>
<th>Mouse Virulence</th>
<th>Carbohydrate</th>
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<td>L. seeligeri</td>
<td>1/2B, 4C, 4D, 6B, UN</td>
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<td>-</td>
<td>+</td>
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</table>

* - UN = undefined.
<table>
<thead>
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<th>Species</th>
<th>Staph. aureus</th>
<th>Rhod. equi</th>
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<tbody>
<tr>
<td>L. monocytogenes</td>
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<td>-</td>
</tr>
<tr>
<td>L. ivanovii</td>
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<td>+</td>
</tr>
<tr>
<td>L. innocua</td>
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<td>L. welshimeri</td>
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</tr>
<tr>
<td>L. seeligeri</td>
<td>+</td>
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</tbody>
</table>
EXAMINATION OF PLATES FOR 
SUSPECT COLONIES

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FOR LIGHT SOURCE YOU CAN USE BAUSCH & LOMB NICHOLAS 
ILLUMINATOR, CAT. NO 31-33-05-28; SEE FISHER 86 CATALOG 
P 1036

REF. LISTERIOSIS, H.P.R. SEELIGER, HAFNER PUBLISHING CO. 
NEW YORK 1961 P. 220
References


Media Section

1. **Blood Agar**

Composed of blood agar base (Oxoid #2) with 7% defibrinated sheep blood. Rehydrate and sterilize as recommended by the manufacturer. Agar and blood should both be at 45-46°C before adding blood and pouring plates.

2. **Carbohydrate Fermentation Broth**

Add BBL purple broth base (15 g) to 900 ml distilled water. Dispense 9 ml to 16 x 125 mm tubes containing a Durham tube. Autoclave at 121°C for 15 min. Prepare all carbohydrates, except esculin, as sterile 5% solutions. Filter sterilize. Add 1 ml carbohydrate solution to 9 ml broth base to yield 0.5% carbohydrate in broth.

Make esculin directly into base broth as 0.5% solution and autoclave 15 min at 115°C.

3. **Enrichment Broth (EB)(pH 7.3)**

TSB-YE (both BBL) supplemented with:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acriflavin HCl (Sigma)</td>
<td>15 mg/l</td>
</tr>
<tr>
<td>Naladixic acid (sodium salt, Sigma)</td>
<td>40 mg/l</td>
</tr>
<tr>
<td>Cycloheximide (Sigma)</td>
<td>50 mg/l</td>
</tr>
</tbody>
</table>

Add the 3 supplementary ingredients aseptically to TSB-YE after autoclaving and just before use. The first 2 ingredients are dissolved in distilled water and filtered for sterilization. Cycloheximide is dissolved in 40% ethanol and water before filter sterilizing.

4. **Modified McBride Agar (MMA)(pH 7.3)**

Phenylethanol agar (Difco) 35.5 g
Glycine anhydride (Sigma) 10 g
Lithium chloride (Sigma) 0.5 g
*Cycloheximide 200 mg 1 liter

Heat to boiling to dissolve agar and dispense into bottles. Autoclave at 121°C for 15 min. Store in refrigerator. Melt and temper agar and then add *cycloheximide (filter sterilize) before pouring plates. These plates may be stored in the refrigerator for 1 week. Do not dry plates.

5. **MR-VP Medium (M92)**

Use either BBL or Difco prepared as recommended by manufacturer. When autoclaving at 121°C, do not exceed 15 min. MR-VP reagents are described below.

Methyl red - 0.1 g methyl red in 300 ml 95% ethanol made up to 500 ml in distilled water.

a-naphthol solution - 5% a-naphthol in absolute ethanol

KOH solution - 40% KOH in distilled water
6. Nitrate Reduction Medium - Nutrient Broth with 1.0 g/l Potassium Nitrate (M96)

Reagent A - Dissolve 0.8 g sulfanilic acid in 100 ml 5 N acetic acid by gently heating.

Reagent B - Dissolve 0.5 g α-naphthylamine in 100 ml 5 N acetic acid by gently heating.

Zinc powder

7. SIM Motility Medium (BBL)

Rehydrate and sterilize according to manufacturer's instructions. Add 6 ml medium per 16 x 125 mm screw-cap tube.

8. Triple Sugar Iron Agar (TSI)(M134)

Use either BBL or Difco prepared per manufacturer's instructions. Streak and stab butt.

9. Tryptose Broth and Agar for Serology

Difco Bacto Tryptose 20 g
Sodium chloride 5 g
Dextrose 1 g
Agar (leave out of broth formula) 15 g
Distilled water 1 liter

For agar, make generous slants.

10. Trypticase Soy Agar with 0.6% Yeast Extract (TSA-YE)

Trypticase soy agar (BBL) 40 g
Yeast Extract (BBL) 6 g
Distilled water 1 liter

11. Trypticase Soy Broth with 0.6% Yeast Extract (TSB-YE)

Trypticase soy broth (BBL) 30 g
Yeast extract (BBL) 6 g
Distilled water 1 liter

12. Urea Hydrolysis Medium

Dissolve 1.5 g agar in 90 ml distilled water and heat to boiling to dissolve agar, then autoclave at 121°C for 15 min. Temper agar to 50-55°C and aseptically add the contents of one 10 ml tube of urea agar concentrate. Mix thoroughly and dispense to sterile tubes and slant generously.